

# COMPOSITION AND PROPERTIES OF GOAT'S MILK AS COMPARED WITH COW'S MILK

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## INTRODUCTION

Goat's milk is used for human consumption through both choice and necessity in many parts of the world. It serves as the chief source of milk in some countries. In the United States the 1930 census reported more than 1,000,000 milk goats.

Goat's milk is used either without consideration of any special characteristic it may possess or because of its specific properties. Some persons who manifest allergic reactions to the ingestion of cow's milk have no such difficulty with goat's milk. The apparent ease of digestion of goat's milk has been attributed to the character of its curd, aided to some extent by the small size of its fat globules. On the other hand, there has developed an impression, gained largely from earlier foreign literature, that children fed goat's milk develop anemia, claimed by some to be due to a vitamin deficiency in goat's milk and by others to a high molecular fat-acid content.

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Reliable data on the composition and properties of goat's milk are surprisingly meager. Although much published opinion exists regarding its nutritional value, controlled experimental evidence supporting such opinion is generally lacking. To obtain such information, therefore, the writers carried on experimental work with goat's and cow's milk at the Agricultural Research Center, Beltsville, Md., from 1929 to 1932, inclusive. The results of the work are contained in this bulletin.

## REVIEW OF LITERATURE

### COMPOSITION OF THE MILKS

Variations in the composition of milk of different breeds of goats have been pointed out by a number of workers. Barbellion (12)<sup>3</sup> compared a number of breeds fed on a similar dietary regimen. He concluded that the Maltese goat gave the richest milk; then in decreasing order he ranked the Spanish and Swiss breeds. The range in fat content was from approximately 2.4 to 5.0 percent and in total solids from 10.0 to 14.7 percent.

Crépin (32) has compiled an extensive series of both individual and collective analyses on the milk of goats of different breeds. The milk of the Nubian goat had an especially high fat content, averaging 6.3 percent, although the protein content was lower than that of the Maltese. The milk of Pyrenees, Massive, Spanish, Syrian, Sudanese, Angora, and Tibetan goats had an average fat content ranging from 4.0 to 4.4 percent and a protein content ranging from 2.6 to 3.7 percent. The Swiss or alpine breeds, including the Toggenburg and Saanen, had an average fat content of 3.7 and a protein content of 2.7 percent. There is considerable variation in the fat content of cow's milk, as shown by Nystrom (99). He found that for six dairy breeds in the United States the fat content ranged from 3.4 percent for the Holstein-Friesian breed to 5.36 for the Jersey.

Lusk (81) pointed out that the principal cause for the more pronounced effect of the feed on the composition and yield of goat's milk than on that of cow's milk is that the goat produces more milk for its weight and is thus more dependent on the food supply. Lusk found that starvation of goats caused an abrupt drop in yield and a rise in fat content of the milk. He further stated that a fat-poor diet fed to sheep and goats produced a milk low in fat, whereas additions of fat up to 1 g per kilogram of animal weight increased the fat content of milk. The increase in the protein content of the diet increased the yield but not the fat content of the milk.

Bender and Maynard (18) recently reported experiments on lactating goats in which a decrease in yield of milk and fat resulted when the fat content of the ration was reduced to 1 percent by extraction with benzene, whereas addition of linseed or coconut oil tended to restore the yield to the original level on natural fat-containing feeds. Maynard and McCay (90) obtained comparable results with cows when the fat content of the grain ration containing 6 to 7 percent was reduced to 1 percent by extraction.

At least four milk proteins have been described: Casein, globulin, albumin, and an alcohol-soluble protein. Wells and Osborne (132) showed that these four proteins are immunologically distinct. Casein,

<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 66.

which is present in the largest quantities and is the best known of the proteins because of its characteristic curd formation under the influence of rennin, makes up about 3 percent of cow's milk. The cascins of the milks of the different species appear to be very similar. Dale and Hartley (35) were unable to show any clear disparity of antigenic properties among different caseins. Although chemical differences are not demonstrable by ordinary methods, a study of the products of racemization, in the hands of Dudley and Woodman (40), yielded evidences of structural differences between sheep's casein and cow's casein, although the amino acid composition of the two caseins appeared to be identical.

The remaining proteins of milk are in the whey. In the results of the usual analyses of milk these proteins are recorded as albumin or whey proteins. These proteins are distinct from casein, both immunologically and chemically. Very little study of the whey proteins in goat's milk has been made. Crowther and Raistrick (33) showed that cow's albumin, whether prepared from colostrum or from milk, is the same but is markedly different from the serum albumin of cows. The globulin likewise is the same whether prepared from colostrum or from milk. It is, however, identical with cow's serum globulin. Globulin may constitute 6 to 12 percent of colostrum. On the other hand, according to Crowther and Raistrick (33), it constitutes only about 0.05 percent of cow's milk. Osborne and Wakeman (101) found the alcohol-soluble protein only in exceedingly small quantities.

The composition of the fat in goat's milk has not been studied extensively. From analyses which have been made it is evident that the composition of milk fat of cows and that of goats are not greatly different. There is evidence that the volatile acids are somewhat higher in the milk fat of goats. This is reflected particularly in increased values for the saponification number and the Polenske number. Bardisian (13) pointed out that the milk fat of human beings is characterized by a high content of unsaturated acids and a small content of low molecular weight acids. This investigator also noted that the milk fat of cows resembled that of humans more closely than did the milk fat of goats. Data quoted by the associates of Rogers (7) show that goat's milk fat is characterized by relatively high caprylic and capric contents and low oleic and linoleic acid contents.

The size and distribution of fat globules in cow's milk have been fairly well established by several workers. Associates of Rogers (7) and Campbell (28) have given comprehensive reviews of this subject, which show the fat globules of cow's milk to have an approximate average diameter of  $3\mu$ . Work by Schultz and Chandler (111) shows that 57 percent of the fat globules in goat's milk are smaller in diameter than  $2\mu$ , whereas Pizzie, as quoted by Richmond (104), shows that the globules have a "medium" diameter of  $1.8\mu$ . Milk possessing small fat globules has been advocated by many pediatricians as an aid in digestion.

#### MINERAL AND VITAMIN CONTENTS OF THE MILKS

In the European literature, frequent reference is made to observations of cases of anemia in children fed goat's milk. Stoeltzner (123) stated that the high content of volatile fat acids in goat's milk has a

definite effect on the development of anemia. Rudder (107) also suggested that the anemia which he observed in infants fed exclusively on goat's milk was due to hemolysis produced by the fat acids of goat's milk when absorbed into the blood stream. Behrendt (16) observed anemia in a large number of infants consuming goat's milk and suggested a vitamin deficiency in the milk as a cause that could be remedied by a more liberal supply of green feed to the goat. Baar (9) differentiated alimentary anemia from several forms, among which were iron-deficient and goat's-milk anemias. Both Baar (9) and Mayr (91) suggested that faulty feeding methods, rather than abnormal inherent qualities of the goat's milk, were responsible for anemia in infants.

It appears that the interpretations of the work of these investigators are subject to modifications in the light of recent advances in the study of anemia and the effects of a milk diet. Recent work by Holt and coworkers<sup>4</sup> with babies relative to the production of hemolytic anemia shows that the goat's milk fat produced no destruction of blood as judged by the urobilin output.

Since the discovery of Hart and coworkers (60) that the addition of copper and iron salts to a milk diet enabled growing rats to maintain a normal hemoglobin level, much of the difficulty in the successful rearing of rats as well as other mammals on a milk diet has been overcome. Hart and coworkers (58) cured anemia in suckling pigs by the administration of iron salts. Mackay (82) found that in bottle-fed babies that were anemic between the third and fifth months of age, but that were otherwise healthy, the anemia could be corrected to a large extent by the administration of iron. Rose and coworkers (106) concluded that in the case of young children 0.5 mg of iron per 100 calories in the diet was sufficient for maintenance only and recommended 0.75 mg of iron per 100 calories for growth and maintenance. The successful treatment of secondary anemia in children and adults with iron and copper therapy is discussed by the Wisconsin Agricultural Experiment Station (135). Observations were made at both the Madison public health centers and in the General Hospital at Montreal, Canada.

Recent work at the Wisconsin Station (136) has revealed that emaciation and related symptoms of faulty growth in experimental animals fed goat's milk supplemented with iron and copper were corrected by a change in the source of supply of the forage. Thus it appears rather certain that the anemia produced in animals fed goat's milk, which has been claimed to be of the pernicious type, is amenable to the same treatment as the nutritional anemia produced in animals fed cow's milk or other diets deficient in iron and copper.

A review of work on the iron and copper content of cow's, goat's, and human milk shows no outstanding or consistent differences among the three kinds of milk. Lésne, Clément, and Zizine (79) concluded that the milk of the human, the cow, the goat, and the ass were all deficient in iron. Waddell, Steenbock, and Hart (127) stated that milk supplemented with small quantities of iron and copper was still not a perfect food. Later, the importance of manganese was shown by Skinner, Peterson, and Steenbock (115), who added this element along with iron and copper to a milk diet with a resulting stimulation

<sup>4</sup> Personal communication.

of the growth of young rats, especially those without adequate stores of manganese when the experimental milk diet was first given.

Elvehjem, Herrin, and Hart (44) increased the iron content of the ration of both cows and goats without the detection of any increase in the iron content of the milk. However, they noted fully 100-percent variation among individual cows. Henriques and Roche (61) found no change in the iron content of the blood of human mothers and of goats when ferrous salts were added to the diets. They were also unsuccessful with intravenous injections of iron in goats. The addition of copper salts to the diet likewise caused little, if any, change in the quantity secreted in the milk in studies made by Elvehjem, Steenbock, and Hart (46).

Milk contains a large number of elements not all of which, in the average diet, are recognized as necessary for the growth and well-being of the animal. The elements that constitute the greater portion of the ash of milk include calcium, sodium, potassium, magnesium, phosphorus, chlorine, and sulphur. Other elements that are present are iron, copper, iodine, manganese, and zinc. Spectrographic studies on cow's milk, by a number of workers, reviewed by the associates of Rogers (7), showed in one or more instances the presence of titanium, aluminum, boron, vanadium, rubidium, lithium, strontium, silicon, chromium, lead, tin, and germanium.

Bosworth and Van Slyke (19) compared the content of the various salts in cow's, goat's, and human milk. Their results show differences in distribution although they were unable to assign any special properties to the individual salts. They cited the presence of both dicalcium and tricalcium phosphate in goat's milk and only dicalcium phosphate in cow's milk. Daniels and Stearns (36) suggested such differences as possibly responsible for the finer curds of goat's milk. Bosworth and Van Slyke (19) also found that chlorine occurred in relatively large quantities in goat's milk, not only as calcium chloride, the form common to both cow's and human milk, but also as the sodium and potassium salts. Stockreiter (122) showed that the quantity of chlorine increases with advance in the lactation period of the goat from approximately 100 to 190 mg per 100 cc of milk.

Although vitamin studies on goat's milk have generally shown no marked differences from those on cow's milk, the studies made have been limited. Few, if any, reports on goat's milk have given a complete picture of the vitamin A, B, C, D, E, and G content from a large herd kept under well-controlled feeding conditions for an extended period.

Goat's milk is practically colorless and contains only traces of the carotenoid pigments. Cow's milk, on the other hand, is generally well pigmented with carotene, the precursor of vitamin A. There has been considerable conflict in the experimental evidence on the relative quantities of carotene and vitamin A as affected by seasonal changes in diet and by breeds. However, a recent report by Baumann, Steenbock, Becson, and Rupel (15) showed that Guernsey milk contained more carotene than preformed vitamin A, Jersey milk approximately equal proportions, whereas in the Holstein milk the carotene constituted approximately 30 percent, and the vitamin A, 70 percent, of the total biologically active vitamin A. The maximum average carotene content was 17 micrograms per gram of Guernsey butterfat produced on pasture, and the minimum was 4.3 micrograms

per gram of Holstein butterfat produced on winter feed. Apparently a lighter-colored butterfat, such as is obtained from Holstein milk, indicated a more thorough conversion of carotene to vitamin A. Also, the difference apparently existing between the pigmented cow's milk fat and the colorless goat's milk fat appears to be due to the same cause. A recent report by Fasold and Heidemann (52) indicated that thyroxine plays an important role in the conversion of carotene into vitamin A. These authors found that thyroidectomized goats secreted large quantities of carotene but no preformed vitamin A. The conclusion was that the rate of metabolism was the governing factor.

Among the investigations on the content of the vitamin B complex in goat's milk, Gunderson and Steenbock (56) observed no difference in the milks from Toggenburg goats and from Holstein, Guernsey, and Durham cows. Their experiments also indicated that the addition of feeds to a diet rich in vitamin B had no effect on the vitamin content of the milk. Abderhalden (2) stated recently that goat's milk is richer than cow's milk in vitamin B.

Most of the reports on the vitamin C content of goat's milk have included a comparison with cow's milk. These reports fail to show any unanimity as to the relative potency of the milks. Although work of Hunt and Winter (74) indicated that goat's milk may be somewhat richer than cow's milk, de Ruyter de Wildt and Brouwer (108) found approximate equality, and Frank (54) found goat's milk to be much less potent than cow's milk. Meyer and Nassau (92) also reported that goat's milk was deficient in vitamin C.

Considerable work has been done with lactating goats in experimental studies with vitamin D. Steenbock and associates (121) showed that milk produced by goats confined in a dark basement did not induce healing of rickets in rats receiving 12 cc of the milk. When the goats were irradiated, 4 cc of milk was effective. Cows under similar conditions did not show the same response in increase in potency of the milk (120).

Studies on the vitamin E content of milk have been confined chiefly to cow's milk in the form of whole and skim-milk powders. Evans, Burr, and Althausen (50) concluded that liquid milk is low in vitamin E and generally inadequate for normal reproduction in rats. However, Anderegg and Nelson (6) reported as little as 15 percent of whole-milk powder to be effective in preventing sterility in female rats.

With respect to vitamins, breed comparisons of cow's milk have been made. The vitamin A content of the butterfats of Holstein, Ayrshire, Jersey, Dutch Belted, and Devon cows showed little if any difference according to Holmes (71). Likewise, no significant difference was found in the milk of Holstein, Ayrshire, Jersey, and Guernsey cows in studies by Davis and Hathaway (38). The lack of difference among breeds in the vitamin B complex has been noted. Dutcher and coworkers (42) found no demonstrable difference in vitamin C in the milks from Jersey and Holstein cows. Although little emphasis has been given to breed comparisons, much of the general knowledge of the vitamin content of milk has been gained from investigations on cow's milk. The influence, on vitamin content, of dietary factors in the production of cow's milk and of heat treatment in the marketing and handling of milk has become well established and needs no further discussion.

## DIGESTIBILITY OF THE MILKS

There is a prevailing opinion that goat's milk is more easily digested than milk of some other species, particularly cow's milk. The foreign literature contains clinical evidence to support this opinion. Mayr (91) reports noteworthy success in the feeding of goat's milk to ill infants. Sparapani (118) points out the value of goat's milk in the treatment of intestinal troubles because of its ease of digestion. He further cites its use by a sanatorium in the treatment of digestive troubles and advocates mixing cow's and sheep's milk with it.

As shown previously, it appears from the work of several investigators that goat's milk has comparatively small fat globules, which many pediatricians regard as an aid in digestion. The buffer capacity, including the hydrogen-ion concentration, and the physical character of the curd formed by the coagulation of the milk are also held to be important factors in its digestibility.

There is considerable variation in reported values for the hydrogen-ion concentration of raw milk. As pointed out by the associates of Rogers (7), differences in values obtained for milk of the same species are due to such factors as differences in methods of determinations, differences in composition of the milks, loss of carbon dioxide from the milk, and perhaps also to the condition of the udder from which the milk was drawn. The pH values reported by Alexander (3) for normal cow's milk range from 6.4 to 6.9 and for human milk from 6.6 to 7.6. Schultz and Chandler (110) give a range of 6.4 to 6.7 for goat's milk.

Buchanan and Peterson (24) calculated the buffer action of cow's milk at several pH ranges and showed that it is greater between 4.5 and 6.0 than at higher ranges. Whittier (134) found the maximum buffer action of cow's milk at pH 5.5. Holm and Webb (70) investigated the buffer capacities of various milks over the pH range concerned in digestion in relation to their suitability as infant foods. The data presented by these workers indicate that goat's milk has a larger buffer capacity and human milk a much smaller buffer capacity than cow's milk in the range of pH 5 to 6. On the other hand, Moser (96) reported a large buffer capacity for cow's milk, a somewhat smaller one for goat's milk, and the smallest for human milk. Watson (130) studied the variations that occur in the buffer capacities of milk obtained from large herds of different breeds of cows. He showed that Jersey milk has a markedly greater buffer capacity than Holstein milk.

In connection with the physical character of the curd, since proteolytic enzymes act on the surface of the protein aggregates, it is evident that digestion will be most rapid when the curd is composed of small particles. Such a coagulum exists in the case of the flocculently curdled human milk. Marriott (86) and Hill (66) showed that raw cow's milk is generally coagulated in the form of a tough, rubbery curd that is not always readily digested by infants. In many cases, according to Brennemann (21) and Courtney (29), such curds have been found in the regurgitated chyme and in the stools of infants.

Jordan and Smith (76) reported that the curds formed in the stomachs of infants fed goat's milk were smaller and more flocculent than in those fed cow's milk. Seventeen of eighteen infants that were not thriving on any other food responded favorably to goat's milk, and for that reason these investigators considered goat's milk to be very desirable for infant feeding. The curd of goat's milk, according to

Hill (68), differs from that of cow's milk in that it is a granular rather than a rubbery type and undoubtedly forms smaller particles in the infant's stomach. He also stated (68) that excellent results have often been obtained with goat's milk in feeding infants and that in many instances goat's milk has been used with success where cow's milk has failed. This fact suggests a possible relationship between curd character and utilization efficiency. On the other hand, Hill (69) has more recently announced that, on the average, goat's milk which he tested had a tougher curd than cow's milk.

Buckley (25), as early as 1914, stimulated research in determining the correlation between digestibility and coagulation when he stated that—

inability to digest and assimilate raw untreated milk from some cows, and the perfect digestion and assimilation of similar milk from other cows by an infant is the experience of nearly all physicians and others having to do with infant feeding. Examination by the usual tests for the cause of this difference in the digestibility of such milks fails to indicate it.

Buckley noticed the wide variation of curd character of various milks when he used dilute hydrochloric acid as a flocculating agent. By this method, he found that human milk failed to flocculate. Goat's milk produced a very slight and finely divided precipitate, but in no case was there any tendency for the formation of the mass produced in cow's milk. Of the latter, he found that Holstein milk had the finest particles and that Ayrshire, Jersey, and Guernsey milks increased in curd-particle size in the order named. This led Buckley to suggest the study of the curd as an index of the digestibility of milk.

Hill (67) in 1923 described a method, now used extensively, that follows the principles of the methods of Buckley (25) and of Allemann and Schmid (4) for the measurement of the toughness of a pepsin-coagulated curd. Hill's method for measuring the curd tension as an indication of digestibility has been substantiated by infant feeding. He reported (68) results of clinical investigations carried out in co-operation with Blood that milks of low curd tension are readily digested by infants that would not thrive on other milks. They concluded that soft-curd milk is of inestimable value to newborn babies, to persistent vomiters of whey and leathery curds, to infantile eczema cases, and to colicky babies, particularly those with indigestion typified by the presence of numerous protein curds in the stools. Their findings indicated that soft-curd bovine milk is tolerated as favorably as human milk, even with respect to assimilation, as shown by gains in body weight. Hill (69) considered curd tension of 30 g as the upper limit of soft-curd milk but that the more desirable milks for infants were lower than 30 g in curd tension. From experiments on dogs and humans, Espe and Dye (47) concluded that hard curds required more time for digestion than soft curds.

A wide range in curd tension is generally found among the individual cows in each breed, though in some breeds the distribution shows a favorable proportion of cows producing soft-curd milk. Hill (68) found that the following percentages of cows in the breeds mentioned had a curd tension below 30 g: Holstein, 7.78; Guernsey, 3.25; and Jersey, 1.73. It has been stated (1) that 15.96 percent of Ayrshire cows tested had a curd tension below 30 g. According to Hill (66), in some cases the physical character of the curd is apparently an

inherited characteristic and a cow producing soft-curd milk in one lactation will continue to do so in subsequent lactations except at the beginning and end of the lactation period. Recent work by Riddell and coworkers (105) indicates a significant daily variation in the curd tension of milk from cows producing soft-curd milk. The stage in the lactation period and breed differences were likewise found to be important factors in influencing curd tension. These authors were also able to show a relationship between curd tension and composition of the milk during the first month following parturition. Weisberg, Johnson, and McCollum (131) concluded that the variations in individual cows were due to variations in concentrations of colloidal caseinates and phosphates.

As an aid to digestion, attempts to obtain a milk of correspondingly softer curd and lower buffer properties have been made principally through chemical and mechanical treatments. According to some reports, the use of such milk has given favorable results. Mojonnier (95) as early as 1905 called attention to the fact that evaporated milk or boiled milk was handled by the infant more nearly like human milk. Marriott (85) used buttermilk and acidified milk for the treatment of sick infants. Later Marriott and Davidson (87) showed that the optimum gastric acidity of normal breast-fed infants was reached in 1½ hours after feeding and maintained for 1 hour. Because of the greater buffer action of cow's milk over the range of hydrogen-ion concentration of the infant's stomach during digestion, acidified milk as routine infant feeding was advocated (88).

Brennemann (22) made a study of the coagulation of cow's milk in the human stomach. He showed that boiled and evaporated milks, buttermilk, and milks to which rennin, limewater, magnesia, barley water, or pepsin had been added, all formed fine curds and were easily digested. Lindet (80) pointed out that boiled milk is not coagulated by rennin unless a small quantity of calcium chloride is added. Heated milks were claimed to have less buffer effect than raw milks owing to the changing of the calcium and phosphates from a soluble to an insoluble form on heating. Sommer and Hart (117) are of the opinion that most cases of coagulation by heat are accelerated by an excess of calcium and magnesium. Hess, Koch, and Sennewald (63) showed that heated milk is more easily digested by pepsin *in vitro* than raw milk, and Wallen-Lawrence and Koch (128) found similar results with trypsin. Washburn and Jones (129) obtained a soft curd and small fat globules by homogenizing cow's milk.

Controlled feeding comparisons, however, have generally failed to show differences in digestibility or in growth response of animals fed on milks known to be unlike in curd character. Washburn and Jones (129) observed no difference in growth of pigs fed Holstein or Jersey milk, nor on homogenized, boiled, or raw milk. They found that the composition of the gain in weight was determined largely by the caloric intake, since the carcasses were fattest in groups fed the milks highest in fat and in calories. The work of Nevens and Shaw (97) with rats shows that raw milks are equal, if not superior, to heated milks in digestibility of fat, protein, and total solids. Their comparisons of the raw milks of five breeds of dairy cows—Ayrshire, Brown Swiss, Guernsey, Holstein-Friesian, and Jersey—showed no significant differences. The apparent digestibility of the fat ranged

from 98.2 to 99 percent, of the protein from 89.7 to 92.3 percent, and of total solids from 91.8 to 93.3 percent. In a study of more than 3,700 children throughout the United States, Frank and coworkers (55) failed to note any significant difference between heated and raw milk, as measured by their growth-promoting properties in children under 6 years of age.

Behrendt (17) reports no marked nutritional difference between goat's and cow's milk. His findings are based on feeding comparisons with infants in two institutions located near each other. In one of the institutions 70 infants were fed goat's milk, and in the other 70 infants were fed cow's milk. All other conditions were similar. At the end of a year's observation, Behrendt concluded that goat's milk is the equal of cow's milk as an infant food.

In metabolism experiments with infants fed cow's and goat's milk, Daniels and Sterns (36) found that the nitrogen excretion was higher when goat's milk was fed, whereas no difference was noted in the calcium and phosphorus balance between the two milks. The need of a generous supply of minerals and of energy-producing constituents in the milk diet of goat kids has been shown by Telfer and Crichton (124). Of a series of four groups of kids reared from birth on whole or modified goat's milk, the group on milk diluted with equal parts of water but supplemented with albumin, lactose, and fat made the best growth, whereas the group on whole milk alone ranked second. The best bone development occurred in the group on diluted milk generously supplemented with a salt mixture.

#### MICROBIOLOGY OF THE MILKS

The microbiology of cow's milk, including the bacterial flora of the udder, has been extensively investigated. Associates of Rogers (7) present a comprehensive review of the subject. These authors list four groups of bacteria that generally occur in healthy udders—staphylococci, streptococci, diphtheroids, and the *Brucella melitensis* and *Br. abortus* group. Dorner (39) used the loop-smear technique proposed by Burri (27) for examining milk aseptically drawn from 132 cows in six herds. By the use of this method, Dorner obtained a calculated herd average bacterial count of 7,475 per cubic centimeter of milk. Among the samples analyzed, *Bacterium lipolyticum*, belonging to the diphtheroid group, was the most frequently encountered organism, followed in order by the streptococci and micrococci groups. When the standard agar plate method was applied to these milk samples, a calculated herd average bacterial count of only 2,775 per cubic centimeter was obtained, and the order of frequency was reversed with which the same three groups of bacteria were encountered. Breed (20) identified 12 species of micrococci from aseptically drawn cow's milk. The average bacterial count of the milk from the udders of the cows examined was in excess of 500 per cubic centimeter.

Aside from contributions in connection with studies of Malta fever, little attention has been given to the bacterial flora of the goat's udder. Since the early work of Bruce (23) and, later, of Eyre and coworkers (51), goat's milk has been considered as the chief source of Malta fever infection. In 1918, however, Evans (48) called attention to the morphological and serological similarity between the micro-organism responsible for Malta fever (undulant fever) in man and Bang's disease in cattle. She was of the opinion (49) that these

two micro-organisms were varieties of the same bacterial species, *melitensis*, and suggested for this group the generic name *Brucella*, also referred to as *Alcaligenes*. These findings, together with reports of many cases of undulant fever not traceable to the use of goat's milk, immediately focused attention on probable sources of *Brucella* infection. Cattle and swine as well as goats are now considered as possible disseminators of this disease.

### TEST ANIMALS USED

Young growing rats were used for the determination of the content of vitamins A, B, D, and G of the milks. Mature female rats maintained on a diet deficient in vitamin E were used for measuring the vitamin E content of the goat's milk. Young guinea pigs were used in the vitamin C tests on both the goat's and cow's milks. Rats and kids were used in experiments to compare the nutritive properties of the milks for growth and well-being. The experimental work with rats, guinea pigs, and kids was conducted in the Beltsville laboratories.

In addition to these studies, records were kept at the Florence Crittenton Mission of babies fed the goat's and cow's milks under the direction of medical staff members of the Johns Hopkins Hospital.

### SOURCES OF MILK AND METHODS OF HANDLING

The goat's milk used in the present investigation was supplied by the herd of milking does of the Saanen and Toggenburg breeds, maintained for experimental purposes at the Agricultural Research Center,

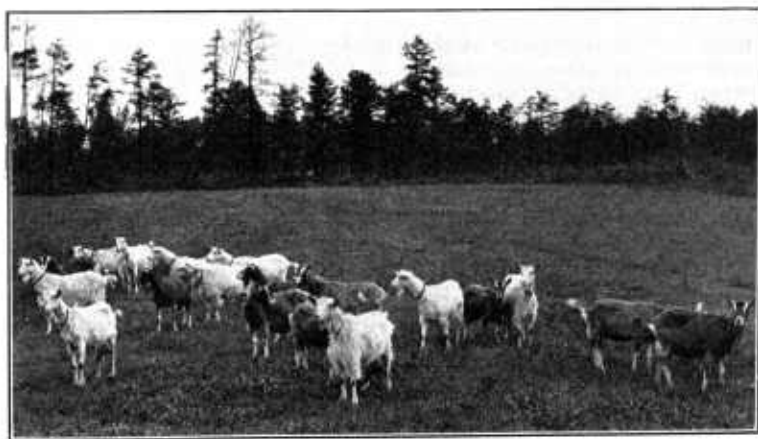


FIGURE 1.—Portion of experimental herd of Saanen and Toggenburg goats.

Beltsville, Md. (fig. 1). The herd contained approximately equal numbers of each breed and was considered representative of milking goats bred to a reasonably high plane of purity. The does from which the milk was obtained varied in number from 20 to 35.

The feeding habits of goats, together with the lack of experimental data on their comparative performance on different diets, made it inadvisable to restrict unduly the experimental conditions necessary for rigid feeding comparisons of goats and cows. Consequently, in

this study data were obtained from goats kept under normal dairying conditions and receiving, for the most part, the same feeds. The winter ration of the does consisted of a good quality alfalfa hay, corn silage, and a grain mixture containing corn, oats, wheat bran, and linseed meal. During the pasture season, green oats and Canada field peas, soybeans, barley, or rye, when available, was grazed in place of the hay and silage in the winter ration.

The cow's milk used was from Holstein and Jersey cattle. The Holstein milk was obtained during 1929 from three cows in a large purebred herd. In 1930 and 1931 a pedigreed herd of 50 to 60 Holstein cows furnished the supply. The ration fed this herd consisted of either alfalfa or clover hay, pasture when available, corn silage, and a grain mixture. The Jersey milk was obtained from three different sources. During 1929, three Jersey cows furnished the supply. In 1930 and 1931, the milk was from representative herds comprising approximately 50 Jerseys. Their feed consisted of alfalfa hay, silage, pasture, and a grain mixture.

The cow's milk was delivered daily from the dairies to the laboratories at Beltsville. Both the Holstein and Jersey milks were cooled to approximately 45° F. either over brine coils or in containers placed in ice water and held at or near this temperature until used. Because of the difference in the composition of these two milks, they were tested separately.

A preliminary chemical comparison of 41 samples of Saanen milk and 48 samples of Toggenburg milk taken at intervals during the lactation period revealed an average fat content of 3.53 percent for the former milk and 3.63 percent for the latter, and a protein content of 3.20 and 3.10 percent, respectively. Consequently in the experiments reported the milks of the two breeds of goats were mixed, as this, as well as other information in hand, indicated little difference between the breeds in the chemical composition of their milks. The morning and evening milks also were combined. The milk was cooled at 45° F. by frequent stirring in a container surrounded by ice water, and this temperature was maintained as nearly as possible by mechanical refrigeration.

The milks used in the infant feeding were sampled for bacteriological and chemical tests, then bottled, packed in suitable iced containers, and shipped daily from Beltsville by truck, arriving within 1½ hours at the Florence Crittenton Mission in Baltimore and not later than 11 a. m. Upon arrival at the mission all milks were immediately used in the preparation of the feeding formulas, were boiled for 1 minute, cooled, and held in the refrigerator until fed.

### CHEMICAL COMPOSITION OF THE MILKS

Daily aliquot samples of the combined milk of the Saaneus and Toggenburgs and also samples of the Holstein and of the Jersey milks were composited and examined weekly for fat, protein, lactose, ash, and water, energy content being expressed in terms of either total nutrients or calories. Iron, copper, calcium, and phosphorus were determined monthly on samples made from aliquots taken from weekly samples. Determinations of iron were made by the mercaptoacetic acid method (78), and of copper by the Elvehjem and Lindow (45) method. All other determinations were made by the methods of the Association of Official Agriculture Chemists (8).

The percentages of fat and protein and the total calories per 100 g of goat's milk based on the weekly analyses between February 1 and November 30 for the 3 years 1929-31 are shown in figure 2. The highest protein content was in February and November 1929, when it reached nearly 4 percent. From May to October of all 3 years, the protein content usually fluctuated between 2.5 and 3.0 percent. On the whole, there was relatively little variation from

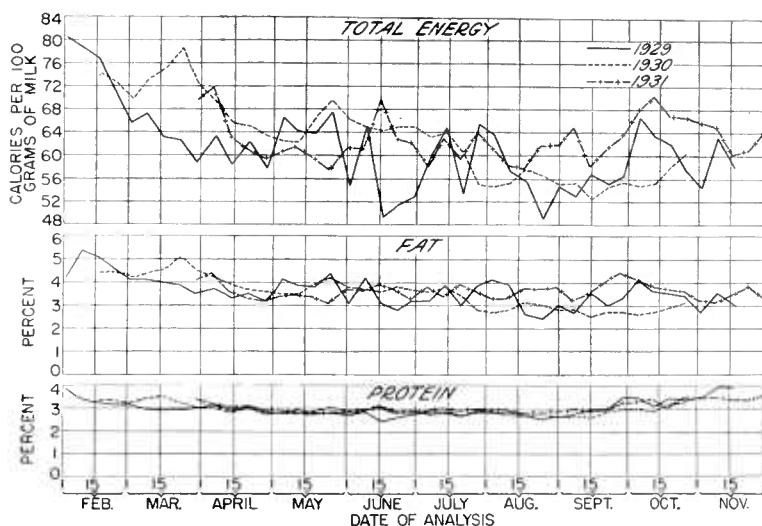


FIGURE 2.—Fat, protein, and total-energy contents of goat's milk, February 1–November 30, average of 3 successive years, during which analyses on daily composite samples were examined weekly.

year to year for corresponding periods. The greatest change was the gradual decline from February to April 15 and then an increase during October and November.

The fat content showed a much greater fluctuation than did the protein. During the 3 years, a value of 5 percent was exceeded only twice. From these maximum values, which occurred during February and March, there was a gradual, although irregular, drop to approximately 3 percent early in September. Unlike the protein, no regular rise in fat content occurred during October and November.

The curves for total-energy content also reflect the changes in protein and fat. The milk produced during August and September was fully 25 percent lower in energy content than a number of the February and March samples. In general, the curves show a consistent and steady decrease in energy content until September, followed by a small increase until the end of November.

Table 1 gives the average monthly composition of the goat's milk (Saanen and Toggenburg combined) and of the Holstein and the Jersey milks. The composition of the goat's milk was similar to that of the Holstein milk; this fact is especially evident in the yearly averages. However, the protein content of the goat's milk from April to September was the lower of the two. Except for one instance, the ash content of the goat's milk was higher throughout the year than the ash content of either the Holstein or Jersey milk.

TABLE 1.—Average monthly composition of goat's milk (Saanen and Toggenburg combined) and of Holstein and Jersey cow's milks (1929-32)<sup>1</sup>

Month <sup>2</sup>	Water			Protein			Lactose			Fat			Ash		
	Goat		Jersey	Holstein		Jersey	Goat		Holstein	Goat		Holstein	Goat		Jersey
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
February	86.95	87.90	85.85	3.39	3.19	3.77	4.91	4.53	4.46	4.3	3.8	5.1	0.82	0.71	0.71
March	87.55	87.98	85.68	3.24	3.09	3.72	4.46	4.58	4.65	4.1	3.7	5.4	0.79	.81	.73
April	87.96	87.96	85.57	2.98	3.08	3.65	4.68	4.68	4.93	3.7	3.7	5.4	.76	.70	.70
May	88.51	87.93	85.69	2.87	3.16	3.72	4.62	4.76	4.90	3.5	3.8	5.4	.76	.68	.70
June	88.81	88.79	85.71	2.88	3.14	3.72	4.34	4.44	4.62	3.5	3.2	5.5	.75	.68	.70
July	88.94	88.49	86.05	2.79	3.10	3.73	4.49	4.54	4.58	3.3	3.4	5.4	.75	.69	.70
August	88.98	88.77	86.09	2.77	3.06	3.69	4.40	4.54	4.59	3.1	3.5	5.2	.77	.69	.73
September	89.20	88.77	85.93	2.57	3.06	3.68	4.40	4.70	4.73	3.1	3.5	5.5	.77	.72	.74
October	88.15	88.05	85.50	3.22	3.18	3.86	4.30	4.70	4.77	3.5	3.5	5.5	.80	.72	.72
November	88.09	88.07	85.61	3.61	3.30	4.08	4.28	4.65	4.75	3.2	3.6	5.5	.85	.76	.76
December	87.64	88.00	84.74	3.53	3.34	4.30	4.75	4.88	4.96	3.5	3.5	5.5	.85	.74	.79
Average	88.29	88.19	85.67	3.10	3.17	3.83	4.55	4.59	4.73	3.5	3.4	5.3	.79	.70	.73

Month	Iron <sup>3</sup>			Copper <sup>3</sup>			Calcium <sup>3</sup>			Phosphorus <sup>3</sup>		
	Goat	Holstein	Jersey	Goat	Holstein	Jersey	Goat	Holstein	Jersey	Goat	Holstein	Jersey
	Milligrams	Milligrams	Milligrams	Milligrams	Milligrams	Milligrams	Milligrams	Milligrams	Milligrams	Milligrams	Milligrams	Milligrams
February	0.02	0.06	0.06	0.060	0.055	0.055	135	112	121	116	85	87
March	0.01	0.06	0.06	0.047	0.050	0.050	126	107	115	107	81	112
April	0.01	0.06	0.06	0.047	0.050	0.050	126	107	115	107	81	112
May	0.01	0.06	0.06	0.047	0.050	0.050	126	107	115	107	81	112
June	0.01	0.06	0.06	0.047	0.050	0.050	126	107	115	107	81	112
July	0.01	0.06	0.06	0.047	0.050	0.050	126	107	115	107	81	112
August	0.01	0.06	0.06	0.047	0.050	0.050	126	107	115	107	81	112
September	0.01	0.06	0.06	0.047	0.050	0.050	126	107	115	107	81	112
October	0.01	0.06	0.06	0.047	0.050	0.050	126	107	115	107	81	112
November	0.01	0.06	0.06	0.047	0.050	0.050	126	107	115	107	81	112
December	0.01	0.06	0.06	0.047	0.050	0.050	126	107	115	107	81	112
Average	0.01	0.06	0.06	0.047	0.050	0.050	126	107	115	107	81	112

<sup>1</sup> Analyses of water, protein, lactose, fat, ash, and total nutrients were made from February to December 1929-31, except for goat's milk, which includes, in addition, February to August 1932. Iron analyses were made in 1929-30, copper from April 1931 to March 1932, and calcium and phosphorus in 1929-31. Except for copper, averages do not include data for 1932.

<sup>2</sup> Because of the small number of goats lactating in January, milk produced during this month was not used in the experimental work.

<sup>3</sup> Results are expressed as milligrams per 100 cc of milk.

The iron content of the milk, irrespective of breed and species, was remarkably constant throughout the experimental period. The Jersey milk showed more irregularity than the others. The content of iron, as determined by the writers, was for the most part lower than that usually given. These determinations leave little room for doubt as to the probability of the development of severe nutritional anemia

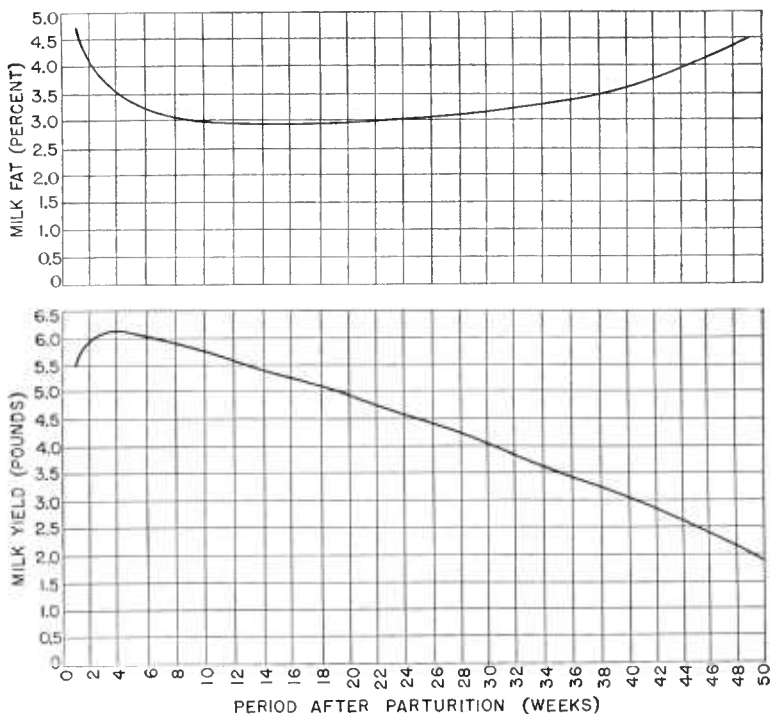


FIGURE 3.—Effect of period of lactation on the daily milk production of goats, as represented by the composite samples of the Saanen and Toggenburg breeds.

in young animals fed on any of the milks alone, and consequently differences in iron content among the milks studied are of little significance.

The copper content was found to be not greatly lower than the iron content. Except for a tendency toward an increase in copper content from February to October in the goat's and Jersey milks, no marked changes or differences among milks were observed. The values obtained in the present work tend to be higher than those of other investigators.

With respect to the calcium and phosphorus contents of the three milks, the Holstein milk was lowest in both elements, and the Jersey and goat's milks were approximately equal. The lowest levels were generally reached in midsummer with a marked rise in phosphorus during September and October.

Although it appears from the data presented that season has a marked effect on the composition of goat's milk, variations due to the

period of lactation are in all probability the principal controlling factors, as shown by figure 3. The figure shows the daily milk production and percentage of fat during the complete lactation period of 13 to 17 Saanen and Toggenburg does whose freshening dates occurred from January 14 to March 21. The data for each animal were obtained for an entire day at regular weekly intervals, beginning 7 days after the animal freshened.

These results indicate a rather rapid decline in the fat percentages of goat's milk in the first few weeks of lactation, the fat content reaching a level that was maintained somewhat uniformly for 18 weeks, after which time a gradual increase in fat content was again observed. During this period the quantity of milk produced increased for the first 4 weeks after freshening, after which a gradual but uniform decrease in production occurred to the end of lactation.

On the basis of these findings, when freshening occurs within a restricted period, as in goats, the tendency for the composition of the herd milk to be typical of the lactation cycle of an individual animal is much more likely than when the freshening dates occur during the entire year, as in cows. The evidence apparently indicates that the marked drop observed in the fat and protein contents of the goat's milk from early spring until late summer was due principally to the fact that nearly all the goats freshened between February and May. Since the samples of Holstein and Jersey milks were obtained from a source wherein the period of lactation was not a factor, because the cows freshened throughout the year, the general chemical composition showed only minor variations from month to month, such as normally occur as a result of seasonal and feed factors.

#### PROTEIN FRACTIONS

In this study determinations were made of the casein, globulin, and albumin in the milks as separate protein fractions. The Holstein and Jersey milks used in the study were obtained during November and December 1932, and the goat's milk was obtained in February of that year. For additional comparative purposes, one sample of human milk was made available for similar analysis through the courtesy of Dr. Park of Johns Hopkins Hospital.

Early in the investigation it became evident that not enough was known regarding the properties of casein and globulin, as they exist in solution in the different milks, to assure the adequacy of known protein methods of analyses when applied to the milks of different species. An extensive study of the methods themselves and their particular application to milk of each species was not feasible. However, some preliminary observations indicated that Moir's method (94) applied to the determination of casein and globulin had noteworthy advantages over the other methods in general use in reproducibility of results, ease of operation, as well as accuracy. This technique, therefore, was used in the present study with some modifications as noted later. There was reason to believe that the actual distribution of protein fractions in the milk was more truly represented by analysis made on skim milk than similar determinations made on whole milk. Accordingly, milk from which the fat had been removed by centrifugalization was used in studying protein partition. All protein fractions were determined as nitrogen and results recorded as grams of nitrogen per 100 cc of skim milk.

Nitrogen determinations were made by the Kjeldahl method. The precipitate resulting from adding 140 cc of 16-percent trichloroacetic acid to 10 cc of milk was analyzed for nitrogen and recorded as total protein nitrogen.

The casein in cow's milk was precipitated at pH 4.5 to 4.55. Because considerable globulin was precipitated from goat's milk by the use of Moir's sodium acetate mixture at pH 4.5, casein was precipitated from goat's milk at pH 4.35 to 4.4. In the case of human milk, better flocculation of the casein precipitate, as well as a clearer filtrate, was obtained when milk was added directly to a 0.2-molar sodium acetate buffer mixture of pH 4.5 than when acetic acid and then sodium acetate were added to the diluted milk according to Moir's method (94).

The filtrate and washings from the casein precipitation were neutralized and sufficient sodium sulphate added to make a final concentration of 1.50 volume molar. The nitrogen in the resulting precipitate was determined and recorded as globulin nitrogen. Albumin nitrogen was calculated as the difference between total protein nitrogen and the sum of casein and globulin nitrogen.

In table 2 are the results of the nitrogen distribution among the proteins of the various milks studied. These data differ from the usual analyses in that they are based on fat-free samples and are expressed in terms of nitrogen without subsequent conversion to protein.

TABLE 2.—*Nitrogen distribution of goat's milk (Saanen and Toggenburg combined) Holstein and Jersey cow's milk, and one sample of human milk*

Source of milk	Weight of nitrogen per 100 cc of skim milk and percentage of total nitrogen in--												Ratio of sum of albumin and globulin to casein
	Casein		Globulin		Albumin		Total protein		Nonprotein nitrogen		Total nitrogen		
	Gram	Percent	Gram	Percent	Gram	Percent	Gram	Percent	Gram	Percent	Gram		
Goat . . . . .	0.3639	70.5	0.0425	8.2	0.0679	13.2	0.4743	91.9	0.0419	8.1	0.5162	0.303	
Holstein . . . . .	.3811	75.7	.0443	8.5	.0467	9.6	.4721	93.8	.0316	6.2	.5037	.239	
Jersey . . . . .	.5167	80.7	.0350	5.5	.0612	9.3	.6129	95.5	.0285	4.5	.6414	.186	
Human . . . . .	.0755	41.2	.0284	15.0	.0496	27.4	.1538	83.6	.0302	16.4	.1840	1.029	

For human milk, Schlossmann, (109) gives the following percentage distribution of nitrogen: Casein, 41; globulin plus albumin, 44 to 39; and nonprotein nitrogen, 15 to 20. Courtney and Brown (30), from an analysis of the milk of 16 normal women, obtained 34.3, 30.5, and 35.2 percent respectively for the three above-mentioned fractions. The data of the present investigators are in good agreement with those of Schlossmann but are somewhat at variance with those reported by Courtney and Brown.

The table shows that the total nitrogen content of goat's milk was similar to that of Holstein milk, a finding that is in harmony with the protein findings shown in table 1. In fact, in the present study, with the exception of the albumin fraction, the nitrogen content of goat's and Holstein milk were similar for all the protein fractions. Jersey milk had the highest content of casein, as a result of which it had also the highest total protein and total nitrogen. The albumin

content of Jersey milk, on the other hand, was slightly lower than that of goat's milk. Human and Holstein milks differed very little in their actual albumin and nonprotein nitrogen contents. The large quantity of nitrogen precipitated from cow's milk as globulin seems surprising in view of the small quantities reported by Crowther and Raistrick (33) and Osborne and Wakeman (101).

On the basis of the percentage of total nitrogen represented by each of the nitrogen fractions, the percentages of albumin and nonprotein nitrogen in goat's milk were somewhat higher than were those in the milk from either breed of cows. In human milk the percentages of nonprotein nitrogen, albumin, and globulin were much higher than were those of either goat's or cow's milk. However, the percentage of casein in human milk was much lower than that in cow's and goat's milk.

Alexander (3) pointed out that as far as nutritional efficiency of milk as a food for the human infant is concerned, the actual concentration of the protein fractions is probably of less importance than the ratios of those proteins that serve as "protective colloids" to the casein. Consequently, the ratio of the sum of the albumin and globulin fractions to the casein fraction of the different milks is included in table 2. These data show that goat's milk, although it has a higher protective ratio than cow's milk, is much lower than the milk of humans in this respect.

#### COMPOSITION OF BUTTERFAT

Six butterfat samples from the Jersey and Holstein milks and from the combined milks of the Saanen and Toggenburg goats were prepared from milks produced in March and June 1931. Fat constants were determined on these samples. In addition, individual fat-acid determinations were made of a sample of butterfat obtained from goat's milk (Saanen and Toggenburg combined) and from cow's milk (Holstein and Jersey combined), both produced during the summer of 1932.

The samples of butterfat were obtained by filtering the decanted melted fat layer from butter churned from sweet cream. Fat constants were determined by methods previously noted (8) except for the thiocyanogen number, in which case the method described by Jamieson (75) was followed. The method of Hilditch and Jones (64) was used for determining fat acids.

Results of the fat-constant study are shown in table 3. Two significant differences in composition of the fats are indicated by these data. (1) Goat's butterfat contains considerably more of the steam-volatile, water-insoluble acids than does Jersey or Holstein cow's butterfat, as indicated by the higher Polenske number and saponification number for goat's butterfat; and (2) cow's butterfat contains the greater number of unsaturated linkages as shown by the higher iodine and thiocyanogen numbers. Since the thiocyanogen number is lower than the iodine number it is considered good evidence of the presence of acids containing more than one double bond.

No material differences in fat constants between the March and June butterfats of any of the milks were obtained. Neither was there any significant difference between the fat constants of the Holstein and Jersey milks. Consequently, in the fat-acid determina-

tions no seasonal distinction of the milks was made, and the cow's milk represented the combined milk of the Holstein and Jersey breeds.

TABLE 3.—*Comparison of fat constants in goat's milk (Saanen and Toggenburg combined) and in Holstein and Jersey milks*

Source of butterfat	Month produced	Fat constants						
		Iodine number	Thiocyanogen number	Saponification number	Hehner number	Reichert-Meißl number	Polenske number	Soluble acids
								<i>Percent</i>
Goat	March	32.5	27.9	234.6	87.8	25.5	7.5	4.8
	June	30.3	26.1	235.4	88.6	26.0	8.1	4.2
Holstein	March	37.4	32.1	228.2	88.5	28.6	2.8	4.4
	June	42.5	36.3	224.3	88.7	27.3	2.2	4.2
Jersey	March	40.2	37.0	225.5	90.0	25.6	2.5	4.3
	June	37.2	35.9	222.6	89.9	27.0	2.4	3.9

Results of the fat-acid study are shown in table 4. These samples of butterfats had the following fat constants: Of the goat's butterfat, the iodine number was 32.2, thiocyanogen number 27.2, saponification number 234.8, and Polenske number 7.6. Of the cow's butterfat, the iodine number was 35.8, thiocyanogen number 30.4, saponification number 228.7, and Polenske number 2.3.

TABLE 4.—*Comparison of the fat-acid content of goat's butterfat (Saanen and Toggenburg combined) and of cow's butterfat (Holstein and Jersey combined)*

#### SATURATED ACIDS

Source of butterfat	Butyric	Caproic	Caprylic	Capric	Lauric	Myristic	Palmitic	Stearic <sup>1</sup>	Total
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Goat	2.4	2.5	1.4	7.2	3.9	10.4	34.4	7.8	70.0
Cow	2.4	1.1	.7	3.6	2.3	12.6	28.5	12.1	63.3

#### UNSATURATED ACIDS

	Decenoic	Tetra-decenoic	Hexa-decenoic	Oleic	Arachidonic	Total
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Goat	0.2	0.4	2.7	25.2	1.5	30.0
Cow	.2	1.1	3.3	30.5	1.6	36.7

<sup>1</sup> Calculated as stearic acid, although very small quantities of higher saturated acids were present.

A significant difference in fat-acid content between goat's butterfat and cow's butterfat is the greater quantities of caproic, caprylic, and capric acids in the former. These acids are the steam-volatile, water-insoluble acids chiefly concerned in the Polenske numbers, and that have been referred to by some workers as having a definite effect on anemia development in infants. These acids may be chiefly responsible for the characteristic odor and taste claimed by some to be prevalent in goat's milk.

The higher iodine number of cow's butterfat can be easily accounted for by the greater quantities of unsaturated acids present, especially oleic acid. Arachidonic acid was present to about the same extent

in both butterfats. This highly unsaturated acid is one of the essential fat acids effective in curing the fat-deficiency syndrome. Although the acid prepared by reduction of the bromides was found to be ineffective by Burr and Burr (26) recent work from several laboratories has shown that the acid prepared by less drastic means is highly effective in curing this pathological condition.

No data have yet been published to determine whether decenoic, tetradecenoic, and hexadecenoic acids have unusual biological significance. Hilditch and Paul (65), however, have suggested a possible explanation of their origin by stating that they may represent degradation products of oleoglycerides that have escaped complete saturation to lower saturated groups.

## DIGESTIBILITY FACTORS OF THE MILKS

### RELATIVE NUMBER AND SIZE OF FAT GLOBULES

Small-sized fat globules have been frequently recommended as an aid in digestion. The fat globules in goat's milk are considered to be generally smaller than in cow's milk. Unpublished work of the authors indicates that the pancreatic enzyme preparation, steapsin, splits the fat of goat's milk more rapidly than the fat of cow's milk, this procedure being due presumably to the smaller fat globules in goat's milk.

In order to establish the relative size and the average number of fat globules in goat's milk and in Holstein and Jersey milks, four examinations of herd samples of each of the three milks were made in November and December 1932 by a modification of Babcock's method (11). A standard hemocytometer was used in measuring the size of globules and a Levy counter for determining the number of globules. Water was used as the diluent, and a technique similar to that used in counting red blood cells was followed. The difficulty experienced in keeping the globules in focus, owing to their tendency to rise, was overcome by insertion of a Whipple eyepiece micrometer disk in the ocular, and the draw tube was so adjusted that the rulings on the counting chamber corresponded with those of the disk. This provided a permanent ruled area regardless of focal depth.

TABLE 5.—*Comparison of the number and relative size of fat globules in the milk of goats (Saanen and Toggenburg combined) and of Holstein and Jersey cows*

Source of milk	Fat content	Fat globules per cubic millimeter	Relative size of fat globules <sup>1</sup>	Diameter of fat globules <sup>2</sup>
	<i>Percent</i>	<i>Number</i>		<i>Microns</i>
Goat.....	3.5	9,200,000	38	1.99
Holstein.....	3.6	4,800,000	75	2.50
Jersey.....	5.4	2,560,000	210	3.53

<sup>1</sup> Calculated by method of Babcock (11).

<sup>2</sup> Calculated by method of Gutzeit (57).

The average results of the examinations are given in table 5. These data indicate that the average relative size of fat globules in the Holstein milk examined was nearly twice as large as the fat globules of goat's milk and that in the Jersey milk they were more than five

times as large. Gutzeit (57), studying the average diameter of fat globules in Holstein and Jersey milks, found values of  $2.58\mu$  and  $3.50\mu$ , respectively. The application of Gutzeit's formula to the data obtained in the present investigation yielded values similar to those he obtained, as shown in table 5. Not only are the data obtained on cow's milk in the present study comparable with those reported by other investigators, but the values for the diameter of the fat globules of goat's milk are in agreement with those of Schultz and Chandler (111).

### SURFACE TENSION

Surface tension is influenced by a number of factors, such as proteins and salts. Studies of the surface tension of the various milks were conducted. The greater surface area of the fat globules in goat's milk, due to the much greater number though smaller size of the former, suggested the likelihood of a difference in surface tension.

Surface-tension measurements were made by the Du Noüy method (41) wherein a chainomatic balance was used to determine the pull required to separate the ring from the milk. Examinations were made of whole milks, skim milks, and milks reconstructed to contain 3, 4, and 5 percent of butterfat.

The surface-tension results expressed as dynes per square centimeter are shown in table 6. These data indicate that no significant difference existed among the three milks either before or after modification, at any level of fat content. Removal of the cream caused a small increase in surface tension. In the series of reconstructed milks, surface tension decreased with increase in fat content.

TABLE 6.—*Surface tension of the milk of goats (Saanen and Toggenburg combined) and of Holstein and Jersey cows at varying percentages of fat content*

[Results expressed as dynes per square centimeter]

Source of milk	Whole milk		Skim milk		Reconstructed milks					
	Fat content	Surface tension	Fat content	Surface tension	Series A		Series B		Series C	
					Fat content	Surface tension	Fat content	Surface tension	Fat content	Surface tension
Goat.....	Percent 3.2	52.0	Percent 0.09	55.9	Percent 3.0	52.5	Percent 4.0	49.8	Percent 5.0	48.6
Holstein.....	3.3	51.5	.08	55.8	3.0	51.7	4.0	49.6	5.0	48.4
Jersey.....	6.7	51.1	.08	55.9	3.0	52.0	4.0	50.6	5.0	49.0

### BUFFER CAPACITY

The hydrogen-ion concentration of the stomach contents plays an important role in digestion. For the stomach contents at the height of digestion in normal infants less than 4 months of age, Davidsohn (37) reported an average hydrogen-ion concentration of pH 5.1 in infants fed cow's milk diluted with water 1 to 3, and of pH 4.9 in similar infants fed cow's milk diluted 2 to 3. Babbott, Johnston, Haskins, and Shohl (10) found a hydrogen-ion concentration of pH 3.2 to 5.0 in the stomachs of normal infants from 3 to 19 months old fed a test meal containing powdered milk. Marriott and Davidson

(87) reported a hydrogen-ion concentration of 3.5 to 5.0, with an average of 3.75, in normal breast-fed infants.

Since the acid-secreting capacity of the stomach of the infant is limited, the buffer capacity of ingested food is of considerable importance. Cow's milk has a higher buffer capacity than human milk over the range of hydrogen-ion concentration concerned in digestion; therefore, cow's milk requires a larger quantity of acid to bring it to the optimum hydrogen-ion concentration for digestion. For this reason Marriott and Davidson (87) advised the acidification of cow's milk when fed to sick infants whose gastric secretion was lessened. These investigators (88) later recommended the use of acidified whole cow's milk as a routine food for infants.

Studies of the buffer capacity of goat's milk (Saanen and Toggenburg combined) and of Jersey and Holstein milks were made during November and December 1931. Four samples of goat's milk were used, two of Jersey, and one of Holstein. The observations were limited to a range of hydrogen-ion concentration between pH 4.5 and 6.5, at which it appeared that differences in buffer capacities would be of greatest significance in the digestion of the milks by infants.

All hydrogen-ion determinations were made with the quinhydrone electrode, as described by Cullen and Biilmann (34). No attempt was made to control losses of carbon dioxide from the milks. The initial hydrogen-ion determination obtained on the four samples of goat's milk ranged from pH 6.54 to 6.57; on the two samples of Jersey milk from 6.62 to 6.66; and on the one sample of Holstein milk the pH was 6.70.

To 50-cc portions of the milk, measured quantities of 0.2 normal hydrochloric acid were added. The acid was added slowly while the milk was stirred by hand. The hydrogen-ion concentration of each portion of milk was determined 2 hours after the addition of the acid.

Titration curves were constructed for each sample of milk, and the quantity of added acid required to produce each increment in hydrogen-ion concentration equivalent to 0.2 pH was read from these curves.

The buffer index  $\frac{d\beta}{d\text{pH}}$  of Van Slyke (126) at the pH range involved

was then calculated for each small increment of acid by the following formula:

$$\frac{d\beta}{d\text{pH}} = \frac{(\text{cubic centimeter of acid added})}{(\text{volume of milk})} \times \frac{(\text{normality factor of acid})}{(\text{pH change})}$$

The values for each  $\frac{d\beta}{d\text{pH}}$  were calculated for each interval of 0.2 pH.

Figure 4 presents buffer curves in which values for  $\frac{d\beta}{d\text{pH}}$  were plotted

against hydrogen-ion concentration. The curves obtained on all samples of goat's milk were so nearly alike, as were those on the two samples of Jersey milk, that only results from one sample of each kind of milk are shown in this figure.

In general, the results obtained on Jersey and Holstein milks are in agreement with those reported by Watson (130). The writers' results on goat's milk, however, differ markedly from those published by Holm and Webb (70), who used only one sample of goat's milk. In the

present investigation, between pH 5.2 and 6.4 the buffer capacity of the goat's milk is very similar to that of Jersey milk. As the hydrogen-ion concentration is increased above pH 5.2, the buffer capacity of goat's milk decreases below that of Jersey milk. Holstein milk has a lower buffer index than either Jersey or goat's milk over the pH range of 4.8 to 6.5.

Whittier (134) has indicated that casein is one of the chief factors in the buffer action of milk at a pH range of 4.5 to 5.7. However, the differences found by the writers between goat's milk and Holstein milk can scarcely be accounted for on the basis of concentration of casein since, as has already been shown, goat's milk has about the same casein content as Holstein milk and a much smaller casein content than Jersey milk.

### CURD TENSION

The determination of the character of milk curds formed by pepsin coagulation, by measuring their resistance to the cutting action of knives as is done in the Hill procedure (66), has come into extensive use within recent years. Application of the test in the present experiments was undertaken to evaluate the curd character of goat's milk, as represented by the combined milk of the

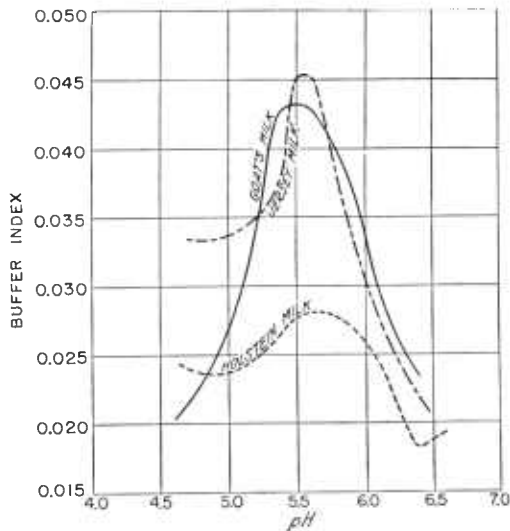


FIGURE 4.—Buffer capacity of goat's milk (Saanen and Toggenburg combined) and of Holstein and Jersey cow's milks at different hydrogen-ion concentrations.

Saanen and Toggenburg breeds, and to compare it with Holstein and Jersey milks. For nearly 2 years, curd-tension studies were made of monthly samples of the various milks. After the studies had been in progress for a short time, a number of questions arose chiefly concerning the use of the calcium chloride in the coagulant prescribed by Hill. The resulting study, particularly as it related to the development of a modified method for curd-tension measurement involving the use of hydrochloric acid in place of the calcium chloride, has been reported by Miller (93).

The modified procedure as finally adopted consisted in the use of a coagulant made up of a 0.4-percent (0.11-N) solution of hydrochloric acid containing 0.45 g of pepsin per 100 cc. The other details of the method were like those given by Hill (66). The apparatus employed consisted of a set of knives having radial blades joined to an upright slender handle, glass jars as containers for the coagulant and milk, a spring balance supported at the top at a convenient height, and a jacketed piston operated with water that provided means of applying

tension to the milk curds by lowering the glass jars at a uniform rate. All tests were conducted in duplicate and when possible in triplicate.

Besides obtaining curd-tension measurements of the milks in the raw state, tests were also made of milks subjected to special treatment, including boiling, homogenizing, separating, and reconstruction by the addition and interchanging of mechanically separated creams. Results are given for only 1 minute of boiling, as boiling for 5, 10, and 15 minutes failed to cause a significantly increased softening of the curd. Homogenization took place at 48° C. under a pressure of 4,000 pounds. Separation was accomplished by passing the milks, at a temperature of 35°, through a hand-operated milk separator. Because of the small-sized fat globules in the goat's milk, it was necessary to run this milk through the separator twice in order to obtain a skim milk with a fat content of less than 0.1 percent. The average curd-tension measurements of the raw and treated milks are given in table 7.

TABLE 7.—Comparison of curd-tension readings of raw and treated milk from goats (Saanen and Toggenburg combined) and from Holstein and Jersey cows, as determined with the hydrochloric acid and pepsin coagulant

Source of milk	Average curd-tension readings of—					
	Whole milk			Skim milk		
	Raw	Boiled for 1 minute	Homogenized at 4,000 pounds pressure	Raw	Boiled for 1 minute	Homogenized at 4,000 pounds pressure
	Grams	Grams	Grams	Grams	Grams	Grams
Goat.....	36	14	21	46	13	25
Holstein.....	52	14	29	59	9	53
Jersey.....	78	33	56	87	36	91

Table 7 shows that the whole raw milk of the goats was 31 percent softer, on the average, than that of Holstein milk and 54 percent softer than that of Jersey milk. The curd tensions of the skim milks were higher than those of the corresponding whole milks. Boiling either whole or skim milks reduced their curd tensions, skim milks being affected to a greater extent by this process than whole milks. This method of treatment resulted in the curd-tension measurements of boiled skim milks being not materially different from the corresponding measurements of boiled whole milks and definitely places such treated goat's and Holstein milks in the class of soft-curd milk. Boiling either Jersey whole or skim milk produced curd tensions representative of the upper limits of soft-curd milk.

The fact that boiling results in lower curd tensions indicates increased stability of the milks and appears to warrant the dismissal of the importance of the albumin as a protective colloid, since lactalbumin is heat coagulable above 70° C. In fact, heating the milks to 70° for 1 to 10 minutes produced no noticeable softening of their curds. That lactalbumin does not function as a thermal stabilizer in milk is borne out by the work of Matsico (89) and the associates of Rogers (7).

It has long been claimed that homogenization produces easily digested milk. By means of the curd-tension test, Weisberg and associates (131) have presented results in substantiation of this claim. In the present work, homogenization at the pressure used (4,000 pounds) was found to soften milk curds but not to the same degree as did boiling. Homogenization failed to lower the curd tension of Jersey whole milk sufficiently to class it as soft-curd milk. Similar treatment of goat's and Holstein whole milks, on the other hand, produced soft-curd milks. Homogenization of skim milks produced no effect on their curd-tension measurements except in the case of goat's milk. Any effect that homogenization may have had on the colloidal structure of the proteins in the skim milk was not reflected in the coagulum formed. The character of the curd seems to be determined by the phenomenon of aggregation of the protein particles, the stability of which apparently is controlled by the ionic balance present in milk.

The fact that whole milks have a lower curd-tension value than their corresponding skim milks suggests that fat may play a part in curd-tension measurements. That fats may differ in this behavior is indicated by the relatively high curd tension of Jersey whole milk, notwithstanding its greater fat content, as well as observed curd-tension differences between goat's and Holstein milk of similar fat composition.

In order to investigate the role of milk fat in curd tension, whole milks and milks reconstructed by the addition to skim milk of mechanically separated creams were tested for curd-tension values. Results are shown in table 8. These include two samples of goat's milk representing a normal curd tension and a high curd tension.

TABLE 8.—*Effect on curd tension of the addition to skim milk of mechanically separated creams, as determined with the hydrochloric acid and pepsin coagulant*

Source of skim milk used <sup>1</sup>	Source of cream added	Fat content of reconstructed milk	Curd tension value
		Percent	
Goat <sup>2</sup> (normal curd tension sample).....	Goat <sup>2</sup> .....	3.2	29
Do.....	do.....	3.6	26
Do.....	do.....	6.6	19
Goat <sup>2</sup> (high curd tension sample).....	do.....	3.0	49
Do.....	Holstein.....	3.0	50
Do.....	Jersey.....	2.3	44
Do.....	Holstein <sup>2</sup> .....	3.3	57
Holstein <sup>2</sup> .....	do.....	5.0	55
Do.....	do.....	3.3	59
Jersey <sup>2</sup> .....	Jersey <sup>2</sup> .....	5.5	76
Do.....	do.....	3.7	81
Do.....	do.....	6.6	80

<sup>1</sup> All skim milks tested less than 0.1 percent of fat.

<sup>2</sup> Original milk before separation (control).

The two samples of goat's milk with varying curd tensions, when separated and reconstructed to the original fat content with cream from either goat's or cow's milk, had the same extreme curd-tension values as the original goat's milk. The same was true in general with Holstein and Jersey milks, in which cream from the milk of

these two breeds was interchanged. Apparently the relative softness or toughness of whole milk curds is primarily a function of the skim milk.

Curd-tension measurements of the milk of 23 individual goats representative of the herd were made at several intervals during their lactation period. In all, 114 samples were measured by the use of the calcium chloride and pepsin coagulant. Of the goats studied, 11 were of the Saanen breed and 12 of the Toggenburg breed.

Table 9 shows the percentage of the goat's-milk samples in various ranges of curd-tension measurements. Comparative data from the milk of Holstein and Jersey cows, taken from Hill (66), are also included in the table. The fact that about 60 percent of the Holstein samples examined by Hill had a curd tension of 50 g or above and about 70 percent of the Jersey samples had a curd tension of 60 g or above is in agreement with the averages presented for these milks in table 7. Likewise, the fact that 55 percent of the samples of goat's milk had a curd tension of less than 30 g is in harmony with previous observations as to the relative soft curd possessed by goat's milk.

TABLE 9.—*Distribution of curd-tension measurements of samples of goat's milk (Saanen and Toggenburg combined) and of Holstein and Jersey cow's milk*

Curd-tension measurements (grams)	Proportion of total number of samples of—			Curd-tension measurements (grams)	Proportion of total number of samples of—		
	Goat's milk (114 samples)	Holstein milk <sup>1</sup> (334 samples)	Jersey milk <sup>1</sup> (263 samples)		Goat's milk (114 samples)	Holstein milk <sup>1</sup> (334 samples)	Jersey milk <sup>1</sup> (263 samples)
	Percent	Percent	Percent		Percent	Percent	Percent
10-19.....	17.6	1.5	0.9	40-49.....	9.6	14.0	12.0
20-29.....	37.8	8.0	1.8	50-59.....	12.1	20.0	9.6
30-39.....	20.3	15.0	4.2	60 and above.....	2.6	41.5	71.5

<sup>1</sup> Taken from Hill (68).

The stage of lactation has been previously pointed out as having an effect on the composition and yield of goat's milk (fig. 3). To determine whether curd-tension measurements were likewise affected, curd tensions were taken of the daily milking of individual does in the herd at regular intervals during lactation. Measurements were made by Hill's (66) method. Figure 5 shows the average results of 91 determinations from 9 Saanen and 8 Toggenburg does during their lactation period. The highest individual average curd tension for this period was 45 g and the lowest 21 g.

Following parturition, as lactation progresses, there is a noticeable decline in curd tension for approximately 12 weeks. For the next 8 weeks the curd tension remains rather uniform, followed by a rise during the latter stages of lactation. This curd-tension trend has a marked similarity to the fat curve under similar conditions (fig. 3). Although the fat content of milk probably plays only a minor role in curd-tension values, fluctuations of this component in goat's milk due to stages of lactation are usually accompanied by similar changes in other chemical components (table 1). It appears,

therefore, that the curd tension of goat's milk varies during the lactation period in accordance with variations in the chemical composition of the milk.

The curd-tension measurements of goat's milk were correlated with the protein and fat contents, Hill's (66) method being used for measuring curd values. Mixed night and morning milks of individual does in the experimental herd were analyzed from March to November. The average analyses of 71 milk samples from 10 Saanen and 10 Toggenburg does were as follows: Protein, 3.14 percent; fat, 3.51 percent; and curd tension, 31.3 g.

By means of scatter diagrams, a study was made of the relationships between a number of the possible combinations of milk constituents

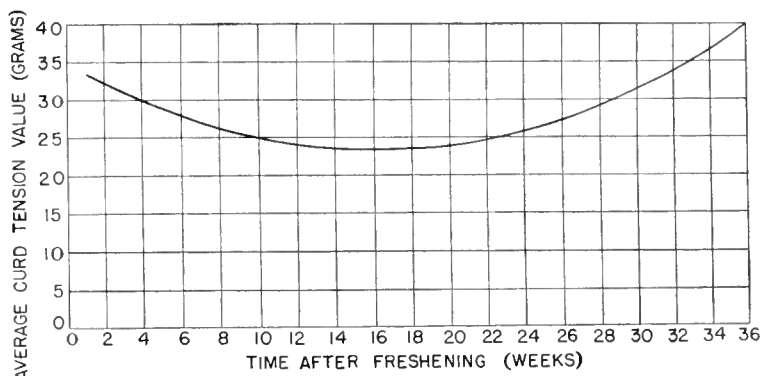


FIGURE 5.—Effect of lactation on the average curd-tension values of goat's milk.

and the curd tension. It was found that a linear relationship existed between the protein content and curd tension. Accordingly, simple, multiple, and partial correlations were obtained for those combinations that appeared to be most critical.

The simple correlation between protein and curd tension of goat's milk was found to be 0.76. Espe and Dye (47) calculated that the correlation between the same factors of cow's milk was 0.88. Although the fat content follows the protein content to some extent, as revealed by a correlation coefficient of 0.61, the correlation of 0.50 between the fat and curd tension shows the low correlation of fat, which was borne out by experiments previously presented, with skim milk alone and combined with various milk fats. Furthermore, the multiple correlation coefficient of 0.74 for curd tension with fat and protein, and a partial correlation coefficient of 0.66 for protein and curd tension with fat, indicate the negligible part that fat plays in determining the curd character.

Nevertheless, analyses of the curds revealed that a high percentage of milk fat was tenaciously retained within the curds. It was found that 94 percent of the milk fat was enmeshed in the curd from Jersey milk, 92 percent in the curd from Holstein milk, and 88 percent in the goat's-milk curd.

Hence, it may be concluded that fat exerts little influence except to prevent the protein from forming an extremely hard curd by the enmeshment of the fat globules, thereby decreasing the cohesive action

of the protein particles and creating points of weakness in the curd. It appears that the character of the curd is relatively independent of the kind or quantity of fat present in the milks and is more or less dependent on the protein and its concentration.

## VITAMIN POTENCY OF THE MILKS

### VITAMIN A

Determinations of vitamin A potency were made on goat's milk (Saanen and Toggenburg combined) and on Jersey and Holstein cow's milk. This investigation was made on summer and winter milks in 1929, 1930, and 1931. Milks produced during June, July, August, and early September were considered summer milks. Winter milks consisted of those produced during February, March, and April. Vitamin tests of butterfats were also made, the milks used being produced in March and June, 1931. The butterfat samples were prepared in the laboratory from sweet cream. After removal of the protein and water, the samples were stored in the refrigerator in brown glass bottles as a precaution against vitamin A losses until the samples were used. The tests were begun after the preparation of the June samples.

The biological rat-assay method of therapeutic testing was used for all vitamin A estimations. This method consisted in feeding to recently weaned rats a diet deficient in vitamin A, composed of the following ingredients, in percentage: Dextrin, 72; purified casein, 18; salt mixture, 4; agar, 1; and irradiated dry yeast cells, 5. This diet was fed for 28 to 42 days, by which time growth had practically ceased and definite signs of xerophthalmia developed. As a therapeutic measure, milk or butterfat was then added to the diet, the milk being fed 6 days per week for 8 weeks at levels ranging from 0.5 to 2.0 cc. The butterfat samples were fed at 21 and 43 mg daily levels, or the equivalent of approximately 0.5 cc and 1 cc respectively in terms of milk testing 5-percent butterfat. Control animals were continued on the vitamin A deficient diet without any therapeutic supplement.

Litter mates were distributed into four groups representing those fed goat's, Holstein, and Jersey milks or butterfats, and controls receiving only the vitamin A deficient diet. So far as possible each of these four groups contained an equal number of male and female rats. Such a distribution of rats, together with the various feeding levels of summer- and winter-produced milks and butterfats, provided a means of comparing vitamin A potencies according to season, sex, and quantity of the therapeutic agent fed.

At the close of each 8-week period, as shown by figure 6, the rats fed Jersey milk had made the greatest average gains on all levels of feeding. Of the 14 groups of rats fed the varying quantities of goat's milk during the 3 years, 7 groups gained more and 7 gained less than the rats fed the Holstein milk. For the entire period, the difference in total gains between the rats receiving the goat's and Holstein milks was not significant. Likewise, for the three winter tests the difference in gains of the rats fed Jersey milk and those fed goat's milk was not significant. The summer tests and the combined summer and winter tests, however, showed a probability greater than 1 to 99 that the Jersey milk contained more vitamin A than the goat's milk. Noteworthy differences were also found between Jersey and Holstein milk.

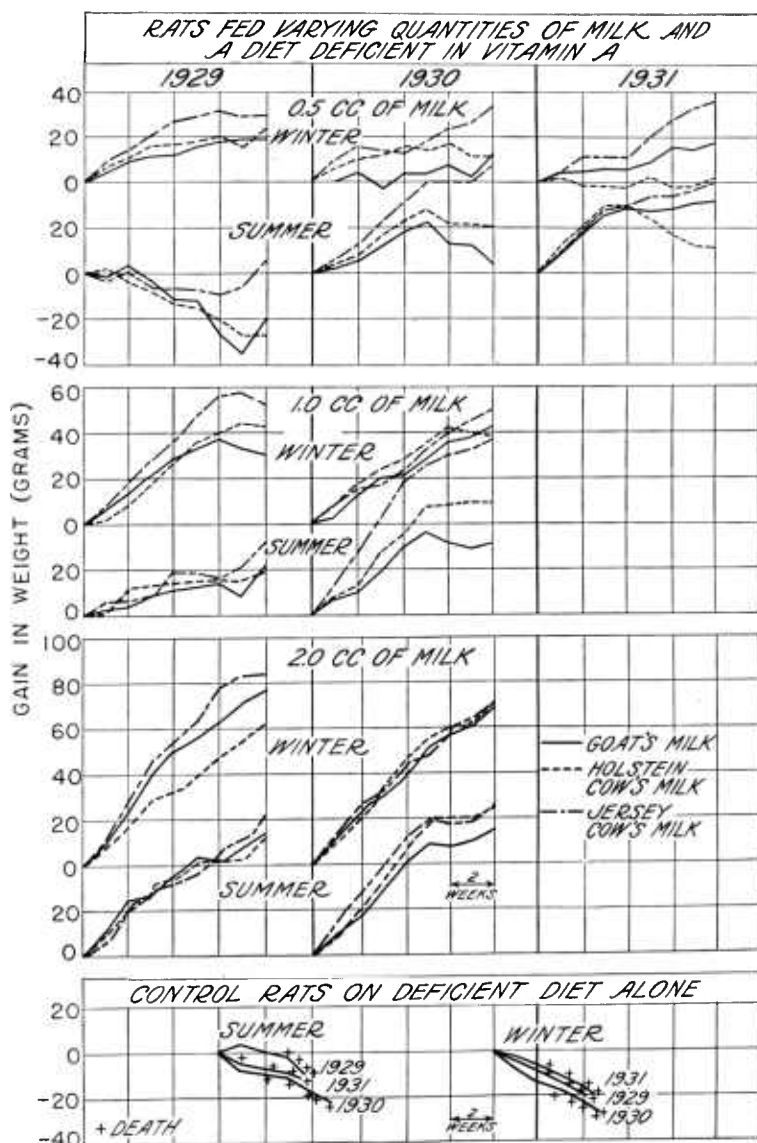


FIGURE 6.—Vitamin A potency of goat's milk (Saanen and Toggenburg combined) and of Holstein and Jersey cow's milk, as shown by gains of rats fed varying daily quantities of the milks and a basal diet deficient in vitamin A for 8 weeks.

The odds were greater than 1 to 99 for the winter milk and 1 to 49 for the summer milk that the Jersey milk contained more vitamin A.

The growth of the rats on the various levels of winter-produced milks generally showed close agreement from year to year. This

point is of especial interest in the case of the Jersey milk, which was obtained from different herds each year. On a feeding level of 0.5 cc, which included the 3 years, the average total gain, for the 8 weeks, of the rats fed Jersey milk ranged from 28 to 36 g. The gains of rats fed goat's milk also showed a close agreement from year to year on this level, the range being 14 to 19 g. The range was considerably greater for the rats fed Holstein milk, the gain being 22 g in 1929 and only 2 g in 1931. On the higher levels of feeding, however, the growth of the rats fed the Holstein milk was in close agreement during the 2 years that the milk was fed. The same uniformity in results were obtained during this time for the other two milks.

The rats on the summer-produced milk failed to show a corresponding uniformity in growth from year to year. In the summer of 1929, the rats grew unusually rapidly during the first 4 to 5 weeks of the depletion period while on the vitamin A-deficient diet alone. Although the control rats, which were allowed to continue on the basal diet alone, showed no unusual rate of decline or survival period (fig. 6), their litter mates on the 0.5-cc dose of milk lost weight during the greater part of the 8-week period. On higher doses, the rats made satisfactory gains.

The results from the summer-produced milks of 1930 are of interest from the apparant parallel they show to the drought conditions which prevailed. The gain curves of the rats fed goat's and Holstein milks show a marked break at about the fifth week of the test, which occurred in mid-July. This period is within 2 to 3 weeks after the time that the pastures became noticeably dry. The feeding of cut green feed or alfalfa hay as substitutes for pasturage in this period apparently failed to maintain the potency of the milks. The effect on the gains of the rats on Jersey milk was less pronounced, owing possibly to the fact that the Jersey herds were in an area less affected by lack of rainfall early in the summer. The curve for the rats on Holstein milk in 1931 also shows a break midway in the therapy period, which was probably related to the diet of the cows.

A summary of average gains in weight of the rats on each milk according to sex of rats, season, and quantity of milk fed is given in table 10. Seasonal averages on goat's milk show a slightly greater vitamin A potency in the winter-produced milk than in that produced in the summer. This finding may be accounted for by the fact that the goat's milk showed a drop of approximately 20 percent in fat content from March to July (table 1), a phenomenon apparently related to the stage in lactation as pointed out previously. Seasonal comparisons of the Holstein and Jersey milks show no such unanimity favorable to either winter or summer milks. Only at the 2-cc levels of milk do the male rats show any consistent superiority over the female rats in their comparative gains in weight on milks produced the same season. When the results from winter and summer milks are combined, the data show that only in the rats fed Jersey milk was there a gain in weight consistently favorable to the male rats at all three levels of milk feeding.

The data show also that whereas 0.5 cc of Jersey milk was generally more than sufficient to produce gains in weight of 3 g per week—the measure of 1 unit of vitamin A as suggested by Sherman and Munsell (114)—upwards of 1.0 cc of Holstein and goat's milk was required for equal growth. The number of International Units of vitamin A per

1 cc of whole milk was estimated as follows: For goat's milk, winter 1 and summer  $\frac{2}{3}$ ; for Holstein milk, winter 1 and summer 1; and for Jersey milk, winter  $1\frac{1}{4}$  and summer  $1\frac{1}{2}$ .

TABLE 10.—Average gains of rats fed a basal diet deficient in vitamin A plus winter- and summer-produced goat's milk (Saanen and Toggenburg combined) and Holstein and Jersey cow's milks

Source of milk	Season in which milk was produced	Sex of rats fed	Data on rats fed indicated quantities of milk daily for 8 weeks					
			0.5 cc		1.0 cc		2.0 cc	
			Rats fed	Gain	Rats fed	Gain	Rats fed	Gain
			<i>Number</i>	<i>Grams</i>	<i>Number</i>	<i>Grams</i>	<i>Number</i>	<i>Grams</i>
Goat	Winter	Male	7	14.9	5	38.4	6	83.8
	Summer		8	4.1	4	22.5	4	66.5
	Winter	Female	4	19.8	2	36.0	4	54.8
	Summer		7	11.9	5	28.2	4	43.0
Holstein	Winter	Male	6	18.7	4	36.3	4	66.3
	Summer		6	13.2	4	44.0	6	67.3
	Winter	Female	4	29.5	2	49.0	3	62.3
	Summer		7	13.6	5	28.6	4	51.3
Jersey	Winter	Male	7	26.0	4	55.7	5	90.2
	Summer		8	43.8	3	77.3	4	75.0
	Winter	Female	5	31.6	2	41.5	4	56.5
	Summer		7	29.7	6	47.5	4	58.5

The responses of the rats to the various levels of milk fed are in general agreement with the results of other investigators. MacLeod and coworkers (84) found that 0.5 to 0.75 cc supplied 1 Sherman vitamin A unit. Dutcher, Honeywell, and Dahle (43) reported that 1 cc of cow's milk from an experiment station herd produced an average gain of 3 g per week, or 1 Sherman unit of vitamin. The comparisons made by Davis and Hathaway (38) of Holstein with Jersey, Guernsey, and Ayrshire milk at 1.0-cc level showed gains of 4 to 7 g per week. The present results, in which gains of 2 to 6 g per week resulted from a 1-cc dose of milk are in agreement with those just mentioned.

The results in table 11 on the vitamin A potency of butterfat for the most part show a considerably greater potency for the summer-produced butterfat than for that produced in the winter. The data also show a greater potency for the winter-produced butterfat of the Holstein cow and the goat than for that of the Jersey cow. The highest mortality among the rats occurred in the groups on Jersey butterfat. The number of International Units per gram of butterfat were estimated as follows: For goat's milk, winter 20 and summer 30; for Holstein milk, winter 20 and summer 30; and for Jersey milk, winter 15 and summer 30.

The results on Holstein and goat's butterfats, like those on whole milk, show approximate equality in vitamin A, not only between the milk of the two species of animals, but also between the summer and winter milks of either species.

Comparisons of the yellow pigmentation of these butterfat samples showed the following order of decreasing intensity of color: (1) Jersey butterfat produced in June, (2) Holstein produced in June, (3) Holstein in March, (4) Jersey in March, (5) goat's in March, and (6) goat's in June. The order of vitamin potency and color intensity even among the samples from the cow's butterfat showed only general agreement.

The lack of color in the goat's butterfat was in no way reflected in the vitamin content, which was comparable to the highly pigmented June butterfats of the Jersey and Holstein cows.

TABLE 11.—Average gains or losses in rats fed a basal diet deficient in vitamin A plus butterfats made from winter- and summer-produced goat's milk (Saanen and Toggenburg combined) and Holstein and Jersey cow's milks

Source of butterfat	Season in which butterfat was produced	Data on rats fed indicated daily quantities of butterfat for 8 weeks			
		21 mg		43 mg	
		Rats fed	Gain	Rats fed	Gain
		Number	Grams	Number	Grams
Goat.....	(Winter.....	1 6	11	6	36
	(Summer.....	6	4	6	61
Holstein.....	(Winter.....	1 6	9	6	27
	(Summer.....	7	19	6	60
Jersey.....	(Winter.....	3 7	25	7	12
	(Summer.....	6	8	7	53

<sup>1</sup> 1 died during seventh week; average for 5 rats.

<sup>2</sup> 1 died during eighth week; average for 5 rats.

<sup>3</sup> 4 died between third and seventh weeks; average for 3 rats.

Distribution of the international standard carotene preparation for use as a vitamin A standard shortly after the conclusion of the tests on the butterfats made advisable a check determination on one butterfat sample. Accordingly, one group of animals was given 1 gamma (0.001 mg) of the international standard carotene preparation daily for 8 weeks. Litter mates of the same sex were allowed a daily dose of 40 mg of goat's butterfat from the March sample. Results showed that the rats receiving the goat's butterfat gained slightly more than 3 g per week, whereas those receiving 1 gamma of carotene gained approximately 5 g. These results from the feeding of 1 gamma of the carotene standard are supported by other unpublished work by the authors, which showed that one-half gamma is insufficient to support growth and 2 gamma is sufficient for growth slightly greater than 10 g per week.

### VITAMIN B

The vitamin B (B<sub>1</sub>) potency of the three milks was compared in a series of four rat-feeding tests, two with winter- and two with summer-produced milks for a period of 8 weeks. The basal diet used in both the vitamin B and the vitamin G experiments consisted in percentage of purified casein, 20; rice or corn dextrin, 65; minerals, 4; agar, 1; and lard, 10. To insure adequate quantities of vitamins A and D, cod-liver oil was fed separately at the rate of 0.5 cc per week. During the entire time, the animals were kept in individual cages with raised screen bottoms. A short depletion period of 10 to 15 days preceded the 8-week experimental milk-feeding period.

Since both vitamins B and G were lacking in the basal diet, it was necessary to add a source of vitamin G for use in the vitamin B assays. Therefore, yeast autoclaved at pH 9 for 2 hours was fed at the rate of 0.5 g daily.

The growth of rats on the basal diet, with or without supplemental sources of vitamins B and G, is shown graphically in figure 7. The

autoclaved yeast had no significant effect in prolonging life as compared with results obtained on the basal diet alone. However, the vitamin B-rich rice polish or extract of rice polish in combination with the vitamin G-rich autoclaved yeast was approximately equal to untreated yeast in promoting excellent growth. On the other hand, the rice polish supplements alone were ineffective owing to the low vitamin G content, whereas yeast was rich in both factors.

Table 12 gives the gains or losses of the rats fed the three kinds of milk in addition to the basal diet supplemented with the autoclaved

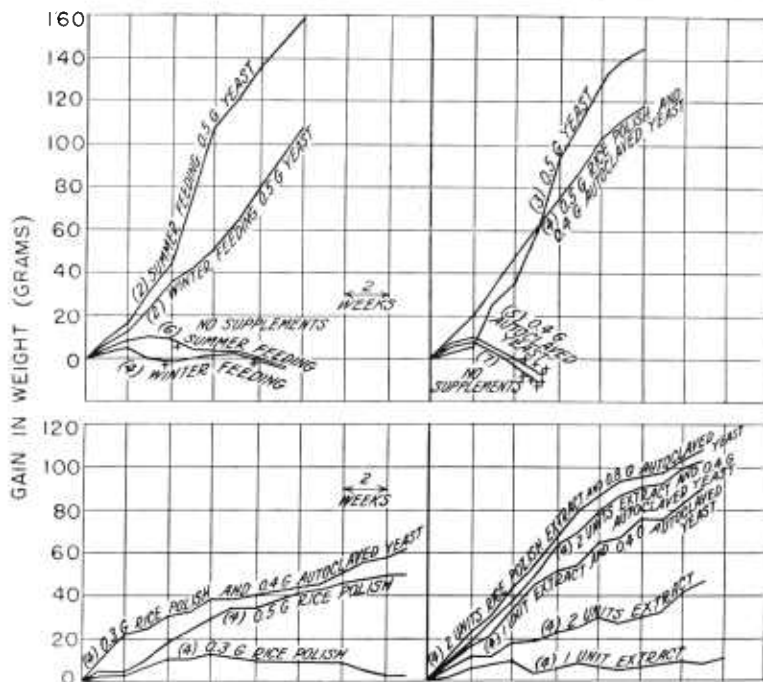


FIGURE 7.—Growth of control rats fed the basal diet with or without vitamin B or G supplement. Figures in parentheses indicate number of rats; + indicates death of rat.

yeast. The table shows that a 2-cc level of winter milk in 1930 was an inadequate source of vitamin B as measured by growth response, whereas the 5- and 10-cc levels fed at this time were adequate. When summer milk was fed at the same levels, greater gains generally resulted on all levels of milk. The 2-cc level of summer milk permitted slow gains in weight, however.

Calculations made according to Fisher's method (53) for significance of difference of means of the gains gave the results shown in table 13. Assuming that the limit of significance is reached with a  $P$  value of 0.05, it is apparent from the table that any vitamin B difference between Holstein and Jersey milk, as reflected by the differences in gain in weights of rats, is not statistically significant. Likewise, between summer- and winter-produced goat's milk there appears to be no sig-

nificant difference, although similar comparisons in the case of cow's milk show significance. Aside from the difference between goat's and Holstein summer-produced milk, which was just outside the limit of significance, differences between goat's and cow's milk appear to be quite significant. Table 13 shows that such comparisons generally favor goat's milk.

TABLE 12.—*Effect of season of production on vitamin B potency of goat's milk (Saanen and Toggenburg combined) and of Holstein and Jersey milks, as measured by growth of rats during an 8-week period in which they were fed these milks and a vitamin B-free diet*

Year and season	Milk fed daily	Data on rats fed—											
		Goat's milk				Holstein cow's milk				Jersey cow's milk			
		Rats fed	Initial weight	Average gain or loss	Average daily consumption of vitamin B-free diet	Rats fed	Initial weight	Average gain or loss	Average daily consumption of vitamin B-free diet	Rats fed	Initial weight	Average gain	Average daily consumption of vitamin B-free diet
		Number	Grams	Grams	Grams	Number	Grams	Grams	Grams	Number	Grams	Grams	Grams
1930:	Cc												
Winter...	2	14	55	—7	3.0	14	53	—4	2.9	14	57	0	3.7
	5	4	54	53	4.3	4	51	24	3.5	4	51	27	3.4
	10	4	59	93	6.7	4	54	87	5.6	4	58	77	5.2
Summer	2	4	66	38	3.9	4	64	28	3.8	4	63	31	3.3
	5	4	65	60	4.4	4	70	45	4.0	4	71	41	3.5
	10	4	63	106	5.8	4	59	74	4.7	4	67	79	4.8
1931:													
Winter...	5	5	68	53	4.8	5	65	34	3.7	5	71	22	3.4
Summer	5	5	62	59	3.8	5	68	52	3.6	5	65	45	3.0

<sup>1</sup> 1 rat died before conclusion of test.

<sup>2</sup> 2 rats died before conclusion of test.

TABLE 13.—*Comparison of vitamin B potency of milks as measured by significance of mean differences in gains of rats*

Groups compared	Mean difference	Value of P	Groups compared	Mean difference	Value of P
Summer and winter milks:			Goat's and Jersey milks:		
Goats (Saanen and Toggenburg combined).....	6.55	0.260	Winter.....	28.78	0.010
Holstein cows.....	19.11	.032	Summer.....	16.44	.030
Jersey cows.....	18.89	.022	Holstein and Jersey milks:		
Goat's and Holstein milks:			Winter.....	5.56	.460
Winter.....	23.22	.013	Summer.....	5.78	.490
Summer.....	10.66	.060			

The number of units of vitamin B in the milk was estimated by comparison with the growth of rats which received the international standard preparation distributed in 1932. The growth on 10 mg (1 unit) daily of this preparation averaged approximately 45 g in 8 weeks. Accordingly, by estimation from the growth on 2- and 5-cc levels, shown in table 12, the goat's milk contained 1 unit of vitamin B in approximately 4 cc of winter-produced milk and 3 cc of milk produced

in the summer. The Holstein and Jersey milks contained somewhat less, the winter milks containing 1 unit of 6 to 7 cc and the summer milk 1 unit in approximately 5 cc.

### VITAMIN G

The vitamin G content of the goat's and the cow's milks was determined in a series of three tests, one during the summer of 1930 and the others in the winter and summer of 1931. In 1930, the test milk was produced in August and September during a drought, whereas in 1931 the summer milk was produced during the same months on relatively green pastures. The rats were kept under the same conditions as those used in the vitamin B tests except that the autoclaved yeast in the diet was replaced with a rice-polish extract prepared with 85 percent of ethyl alcohol. Animals on this basal diet made little or no gain in weight during a 12- to 14-week period, by which time they usually showed marked shedding of hair from the rear quarters, reddened skin in some instances, and emaciation. The inclusion of autoclaved yeast or other vitamin G supplements in the diet permitted normal gains and eliminated or prevented the deficiency symptoms.

The data on the vitamin G content of the milks from the three sources are given in table 14. Only the 5-cc level of milk was fed. Little difference was noted between the summer milks of 1930 and 1931 and between seasons in 1931. Although Hunt and Krauss (73) have noted a decrease in the vitamin G content of milk with advances in summer season and decrease in the availability of tender green forage, the present results did not show any effects of the drought on the ability of the pastures to maintain the vitamin G content of the milk.

TABLE 14.—*Effect of season of production on vitamin G potency of goat's milk (Saanen and Toggenburg combined) and of Holstein and Jersey milks,<sup>1</sup> as measured by growth of rats during an 8-week period in which they were fed these milks and a vitamin G-free diet*

Year and season	Data on rats fed—											
	Goat's milk				Holstein milk				Jersey milk			
	Rats fed	Initial weight	Gain	Daily consumption of vitamin G-free diet	Rats fed	Initial weight	Gain	Daily consumption of vitamin G-free diet	Rats fed	Initial weight	Gain	Daily consumption of vitamin G-free diet
	Number	Grams	Grams	Grams	Number	Grams	Grams	Grams	Number	Grams	Grams	Grams
1930: Summer	4	50	66	5.0	4	51	49	4.4	4	52	56	4.8
1931: Winter	5	71	84	6.6	5	68	58	5.5	5	53	63	5.3
Summer	5	54	72	5.6	5	55	52	5.5	5	54	63	5.0

<sup>1</sup> 5-cc level daily fed in all cases.

Comparisons by means of the *t* test for significance of differences of means relative to the vitamin G content yielded the results shown in table 15. The *P* values on the comparisons of goat's milk with

Holstein milk for both summer and winter tests and for goat's milk with Jersey milk for the winter tests showed significant differences favorable to the goat's milk. No significant difference existed between the other milk comparisons.

TABLE 15.—*Comparison of vitamin C potency of milks as measured by significance of difference of means in gains of rats*

Groups compared	Mean differences	Value of <i>P</i>	Groups compared	Mean differences	Value of <i>P</i>
Goat's and Holstein milks:			Jersey and Holstein milks:		
Winter.....	24.65	0.015	Winter.....	5.00	0.400
Summer.....	18.44	.010	Summer.....	9.00	.270
Goat's and Jersey milks:					
Winter.....	19.65	.010			
Summer.....	9.44	.220			

### VITAMIN C

In the investigations on vitamin C, the combined milks of the Saanen and Toggenburg goats and the milk of the Holstein and Jersey cows were studied in 1929, 1930, and 1931. During each year the winter-produced milk was tested during February, March, and April, and the summer-produced milk during June, July, and August.

The vitamin C potency of milk was determined by feeding experiments with young guinea pigs using the growth method. The guinea pigs were placed on the scorbutic diet when their weights approximated 250 g. This diet consisted, in percentage, of rolled oats, 66; alfalfa leaf meal (autoelaved at 15 pounds of pressure for 30 minutes), 25; casein, 5; irradiated dry yeast cells, 3; and sodium chloride, 1.

The test substance was fed daily to the animals as a supplement to the scorbutic diet. To one group of animals 40 cc and to another group 60 cc of milk were made available in the usual case. The milk was measured into casseroles and the actual daily consumption recorded. Great difficulty attended the various attempts to obtain full consumption at these levels. The withholding of water and the addition of milk in two or more portions daily were without avail. Frequently somewhat more than the required 40 or 60 cc of milk were measured into the casseroles in order to keep the weekly average near the required level. Numerous animals were discarded because they failed to consume the required quantities. Control animals were fed only the scorbutic diet or this diet plus measured quantities of carrots or orange juice. Those receiving the scorbutic diet alone were considered as negative controls, and those receiving in addition the supplements known to be rich in vitamin C were considered as positive controls.

In addition to the keeping of a weekly record of body weight and milk consumption, each animal was examined for scorbutic symptoms. A maximum score of 4 was given to animals in advanced stages of scurvy when they were just able to stand but had lost the power of locomotion in their rear limbs. A score of 1 indicates the initial stage of scurvy as evidenced by tenderness and stiffness of the joints. Most of the animals were autopsied. In this examination, the Sherman procedure (113) was used as a guide in the classification of severity of scurvy. A composite or average autopsy score ranging from 0 to 4

was obtained instead of the additive score used by Sherman. In this manner the clinical and autopsy scores were on a comparable basis. The latter score usually showed more marked scurvy since clinical signs were not always fully evident.

Results of the vitamin C work are shown in table 16. The animals on the scorbutic diet alone developed scurvy within 3 weeks and died within 33 days of the time that they were placed on experiment. Carrots fed at either 25- or 10-g levels furnished full protection against scurvy. Although 1.5 cc of orange juice failed to furnish full protection in all cases, the 3-cc level was adequate and permitted normal gains in the animals.

TABLE 16.—*Effect of vitamin C supplement on body weight and on incidence of scurvy among guinea pigs fed a scorbutic diet for 8 weeks*

Supplement	Year	Data on guinea pigs fed—									
		Winter-produced milk					Summer-produced milk				
		Animals fed	Daily consumption	Gain (+) or loss (—) in weight <sup>1</sup>	Animals dead of scurvy	Average stage of scurvy in survivors <sup>2</sup>	Animals fed	Daily consumption	Gain (+) or loss (—) in weight <sup>1</sup>	Animals dead of scurvy	Average stage of scurvy in survivors <sup>2</sup>
		Number	Cc	Grams	Number		Number	Cc	Grams	Number	
Combined milk of Saanen and Toggenburg goats.....	1929	3	39	+58	0	1.6	3	38	+48	0	1.0
		2	57	+138	0	0	3	55	+107	0	0
		5	38	-22	3	3.0	5	38	-12	1	3.0
	1930	3	49	+101	0	1.0	4	54	+21	0	1.7
		5	40	-31	2	1.7	5	38	-73	5	-----
Holstein cow's milk.....	1931	5	57	+5	0	2.0	3	50	-39	3	2.0
		3	38	-44	3	-----	3	39	+48	0	0
	1929	3	56	+40	1	0	3	50	+170	0	-----
		7	36	-62	7	-----	5	37	-75	5	-----
	1930	6	38	-58	6	-----	5	32	-26	5	-----
Jersey cow's milk.....	1931	5	51	-36	5	-----	4	48	-27	4	-----
		3	39	+29	0	1.0	3	38	+112	0	0
	1929	3	48	+86	0	0	3	53	+31	0	0
		3	38	-23	2	3.0	5	39	+3	1	3.5
	1930	2	47	-31	2	-----	3	47	+9	0	3.7
None.....	1931	4	40	-109	2	3.5	5	35	-56	5	-----
		7	52	-54	7	-----	1	47	-60	1	-----
	1929	3	-----	-60	3	-----	3	-----	-74	3	-----
		3	-----	-69	3	-----	2	-----	-53	2	-----
	1931	2	-----	-95	2	-----	3	1.5	+18	0	1.3
Orange juice.....	1930	3	1.5	-35	0	2.7	3	1.5	+9	1	.7
		3	1.5	+86	0	1.0	3	1.5	+9	0	0
	1931	2	3.0	+60	0	0	3	3.0	+90	0	0
		2	3.0	+122	0	0	2	3.0	+79	0	0
	1931	2	3.0	+122	0	0	2	3.0	+79	0	0
Carrots.....	1929	Grams		+236	-----	0	Grams		+127	0	0
		3	25				3	10			

<sup>1</sup> At death or end of experimental period.

<sup>2</sup> 1 indicates initial stage; 2, mild; 3, severe; 4, advanced.

Goat's milk at the levels fed gave partial protection against scurvy in the usual case; these levels therefore represent border-line doses of vitamin C. Of 13 guinea pigs that consumed approximately 40 cc of winter-produced goat's milk, only 8 survived the experimental period of 8 weeks. Greater consumption of milk, ranging from 49 to 57 cc per day, gave somewhat better protection since all 10 animals survived the experiment. In 1929 both the winter- and summer-produced

goat's milks indicated a somewhat greater vitamin C potency than in subsequent years. During the 3 years a greater variation in potency occurred in the summer-produced goat's milk than in that produced in the winter. The summer milk of 1929 had the greatest potency; that of 1931 had the least. Relative abundance of green forage was probably the most important single factor responsible for the variations.

The results on Holstein milk, as given in table 16, show that the milk in 1929 was apparently more potent in vitamin C than that produced in 1930 and 1931. In 1929 two of three animals that consumed a daily average of 56 cc of winter milk and all animals receiving 50 cc of summer milk survived the test period and showed no scorbutic symptoms. On the other hand, all animals receiving milk produced in 1930 and 1931, which was obtained from the same herd, died from scurvy during the course of the experiment regardless of the quantity of milk consumed. When autopsied, all the animals showed unmistakable signs of scurvy, which appeared to be the main cause of death. Animals on winter milk died nearly as soon as the negative controls. Summer milk furnished slightly more protection since the animals lived longer than on winter milk.

The Jersey milk showed considerable variation from year to year in vitamin C potency. In 1929 the average daily consumption of 39 cc of winter milk furnished moderate protection comparable to that obtained from goat's milk. The summer milk showed a slight increase in potency as judged by the freedom from scurvy of six animals on test. The results of 1930 on animals fed milk from another herd of Jersey cows showed a high mortality of guinea pigs on winter milk and a low mortality on summer milk. However, none of the animals on the latter milk were free of scurvy, four of the seven lost weight, and all showed severe scurvy on autopsy. Hence, the summer milk was little if any higher in vitamin C content than was the winter milk. In 1931, only 2 of 17 animals survived the experimental period and these 2 lost greatly in weight. The average daily consumption of 52 cc of winter-produced milk was not effective in greatly delaying the onset of scurvy, which generally occurred during the second and third week on the scorbutic diet. A consumption of more than 50 cc per day of summer milk was not obtained in any animal, and quantities up to 47 cc per day failed to prevent scurvy.

In view of the wide range in the size of the protective dose of milk against scurvy in guinea pigs as reported in numerous investigations, the present results obtained over a 3-year period are not surprising or unusual. The present experiments were planned primarily to study goat's and cow's milks produced under the feeding and management conditions generally practiced by dairymen and marketed as raw milk. Rainfall apparently was an important factor so far as it influenced the growth of grass and of the corn used for silage. The results, especially those of 1929, generally support the evidence reviewed by associates of Rogers (7), which shows that the vitamin C content of milk from cows on succulent summer pasturage is usually higher than that of milk from cows on dry feeds.

Approximately 50 cc of pasture-produced cow's milk gave full protection from scurvy in 1929. In one instance 38 cc of Jersey milk gave protection in 1929. The same result was obtained with 50 cc of milk by Hart, Steenbock, and Ellis (59), and with slightly less than

50 cc by MacLeod (83), on summer as well as winter milk. As was the usual case, the difference between Holstein and Jersey milks was more striking than the difference between goat's and Jersey milks.

In the two subsequent years, the consumption of 50 cc of Jersey or Holstein milk, produced either in summer or winter, failed to prevent the development of scurvy. Hess and Unger (62) stated that an 80-cc daily allowance of milk is required, and Barnes and Hume (14) found that 100 to 150 cc of raw milk is required for protection against scurvy. In the present study, it was not possible to induce guinea pigs of 200 to 350 g in weight to consume such large quantities. There were only occasional instances of the consumption of 400 cc or more during 1 week. Therefore it was not possible to determine the protective dose.

The wide variations noted from year to year in the results obtained on goat's milk from the same herd and on Jersey milk from three herds make unlikely any pronounced or persistent inferiority of Holstein milk. Because of the variations obtained, a relative ranking of cow's and goat's milk or of milk from different breeds of cows as to vitamin C content for use as a general criterion is not feasible. The low vitamin C content of milk, together with the high demands of the guinea pigs for this factor, tends to accentuate the differences observed among milks. It was not possible, therefore, to assign any ratio or percentage that would indicate, for example, that one milk had one-half or one-fourth the potency of another milk. Since none of the milks were outstandingly high in vitamin C, there can be no question as to the desirability of the addition of an antiscorbutic factor as a supplement to milk.

Subsequent to the completion of the vitamin C assays by the biological method, two developments have taken place in the field of vitamin C research. (1) There was the discovery of the chemical nature of vitamin C, namely, ascorbic acid. (2) A number of chemical methods were developed for the determination of ascorbic acid in biological materials. By the use of modifications of the method originally proposed by Tillmans and coworkers (125) numerous investigations have been made on the vitamin C content of milks of various animal species. Comparisons have also been made of the results, by the biological and the chemical methods, through the intermediary of test feedings of graded quantities of ascorbic acid to guinea pigs. Whitnah and Riddell (133) found that guinea pigs were equally as well protected by a daily dose of 1 mg of ascorbic acid as by 40 cc of fresh raw cow's milk which, on titration, showed a content of 1.04 mg of ascorbic acid.

In order to supplement the data obtained in 1929-31 by the authors, chemical determinations of vitamin C in goat's milk were made at monthly intervals for 1 year beginning in June 1936, the method of Rasmussen and coworkers (103) being used. Milk samples from individual goats were obtained from the morning milkings, taken to the laboratory, and analyzed during the day. The conditions of handling of the milk were kept as nearly as possible like those followed in the earlier guinea-pig tests. Undoubtedly some loss in titratable ascorbic acid occurred in the interval from the milking of the goats to the time of the analysis. The data obtained on the milk from individual goats over the 12-month period ranged from 5 to 25 mg, with an average of 13 mg, of ascorbic acid per liter, or 1 mg in 77 cc.

Although the spring and summer milks tended to have a higher content than that of the fall and winter milks, a 1-mg protective dose (133) was seldom contained in less than 60 cc of goat's milk of any season.

These data aid in the explanation of the vitamin C results previously obtained by the animal-assay method. The guinea pigs used in the biological tests usually consumed from 30 to 50 cc of milk, which is considerably less than the quantity usually accepted as a sufficient protective level. Furthermore, some loss of vitamin C probably occurred after the milk was placed in the cage and before the guinea pigs had consumed their allowance, in addition to that lost from the time of milking to the arrival of the milk in the laboratory. Under such conditions variations in results may be expected.

In order to determine the extent of loss of vitamin C in goat's milk through exposure to air and light, samples were placed in open jars at room temperature. At the end of 20 hours, the loss in ascorbic acid was approximately 40 percent. Selleg and King (112) found that human milk held in a refrigerator for 18 hours lost 27 percent, whereas Krauss and Washburn found, as reported by the Ohio Agricultural Experiment Station (100), that cow's milk lost 50 percent of its ascorbic acid content in 6 hours after pasteurization or boiling.

#### VITAMIN D

The question of the quantity of milk required to induce healing in rachitic rats has led to widely conflicting results by different workers. In numerous instances 4 to 12 cc of milk daily has proved to be sufficient, although Outhouse, Macy, and Brekke (102) have reported that 30 cc was required. Steenbock and coworkers (120) showed that 1 cc of milk from a cow fed a suitable dose of irradiated yeast was more effective in the prevention of rickets than 4 cc of herd milk.

In the present studies, young rats were rendered rachitic by feeding the Steenbock and Black (119) diet for 3 to 4 weeks. The animals were then given eight daily feedings of milk and were killed on the tenth day. The milk was fed in doses of 4, 8, and 12 cc in all tests.

After the animals were killed, the femurs were dissected out for bone analyses and the tibiae for line tests. The latter bones were preserved in 10-percent formaldehyde. Line-test readings were made by at least two members of the laboratory staff, first on one tibia shortly after the rats were killed, and finally on the other tibia after all the tests were completed. In the latter case, comparisons between milks, seasons, and years were given especial attention. The final value reported was a numerical average of the gradings; actually, there was little variation in the various readings within a series.

Beginning with a negative reading as 0, the next reading as 1, and the best calcification denoted by 4, these numerical values on each rat were averaged. Photomicrographs were also taken of selected bones showing typical line tests for the milk and dosage as a basis of reference in the final interpretation of results.

The femur bone was dried, extracted with alcohol, then with ether, and finally ashed. In 1929 these analyses were made on individual rats, and in 1930 one bone of each rat of a group was analyzed for one determination and the other set of bones in a duplicate determination. The results on bone analyses were expressed as percentage of ash of the dry extracted weight of bone. In the interpretation of results these data ranked as secondary to the averaged line tests.

Weight and feed-consumption records were kept weekly and feed consumption was calculated to a daily basis. Data on animals whose abnormal behavior or stunted growth rendered them useless to the experiment were omitted from the final summary.

The average results on control rats fed the rachitogenic diet without supplement and killed at the beginning of the therapy are shown in table 17. All showed a reasonably uniform degree of rickets. Table 18 shows the data on rats that received milk therapy. Examination of the results presented shows that there was no consistent difference in antirachitic properties among goat's milk (Saanen and Toggenburg combined) and Holstein and Jersey ew's milks. Line-test values and percentage of ash agree in this respect. The quantity of milk required to produce healing tended toward the lower rather than the higher levels stated by various investigators. In the present work 4 to 8 cc of summer milk and 8 to 12 cc of winter milk were found to be minimum doses.

TABLE 17.—Average results obtained on control rats fed the rachitogenic diet without supplement and killed at the beginning of therapy

Season and year	Rats fed	Line test <sup>1</sup>	Ash in femur	Season and year	Rats fed	Line test <sup>1</sup>	Ash in femur
Winter:	<i>Number</i>		<i>Percent</i>	Summer:	<i>Number</i>		<i>Percent</i>
1929.....	7	0.53	39.8	1929.....	5	0.70	37.8
1930.....	4	.39	38.4	1930.....	5	.70	40.8

<sup>1</sup> Numerical values represent degree of bone calcification: 0, none; 1, perceptible; 2, distinct; 3, advanced; and 4, nearly complete.

### VITAMIN E

The vitamin E content of the whole milk of goats (Saanen and Toggenburg combined) was studied in the winter of 1933 by feeding experiments with rats. The basal diet, deficient in vitamin E, consisted, in percentage, of alcohol-extracted casein, 20; lard, 15; minerals, 4; agar, 1; yeast, 6; and corn dextrin, 54. Cod-liver oil was administered three times weekly.

Fourteen female rats reared on the diet just described were mated after puberty. After signs of pregnancy had been observed, the animals were examined for the placental sign 12 to 14 days later and then weighed daily throughout the remainder of the gestation period. After resorptions occurred from this initial pregnancy, the females were mated again and fed milk at levels of 10, 25, and 40 cc per day throughout the gestation period. They were examined as before for the placental sign, weighed, and resorption or birth of litter recorded.

No material changes in the weight of the animals were observed. The pregnancies of the rats on the various levels of milk resulted as follows: The four pregnancies that occurred on the 10-cc daily level all resulted in resorptions. In the five pregnant rats receiving a daily level of 25 cc there was no delivery of litters. The five pregnancies on the 40-cc level, the highest daily consumption that could be obtained, also resulted in resorptions. Thus, all the pregnancies studied resulted in resorptions. A group of eight control rats, fed known sources of vitamin E in addition to the basal diet, produced normal litters in all cases.

The data from these studies indicate the absence of detectable quantities of vitamin E in whole goat's milk at the levels fed.

TABLE 18.—Average results of tests on the vitamin D content of goat's milk (Saanen and Toggenburg combined) and of Holstein and Jersey cow's milks produced under winter- and summer-feeding conditions and fed to rats for 10 days

Source of milk	Year	Season	Data on rachitic rats fed indicated quantities of milk														
			4 cc				8 cc				12 cc						
			Rats fed	Gain in weight	Line test 1	Ash in femur	Rats fed	Gain in weight	Line test 1	Ash in femur	Rats fed	Gain in weight	Line test 1	Ash in femur			
			Grams		Percent	Number	Grams		Percent	Number	Grams		Percent	Number	Grams		Percent
Goat	1929	Winter	3	19.0	0.44	39.5	3	26.5	1.22	40.3	3	32.7	1.33	38.6			
	1930	do	2	17.5	1.17	40.0	4	16.5	1.33	41.7	4	21.4	2.30	45.1			
	1929	Summer	4	18.0	1.70	44.9	4	18.5	2.17	44.9	4	22.0	2.30	45.1			
	1930	do	4	11.5	1.55	50.2	4	18.5	2.00	49.3	4	22.0	2.25	51.0			
Holstein	1929	Winter	3	21.5	1.22	42.8	3	26.5	1.05	39.5	3	32.3	1.94	38.9			
	1930	do	2	7.5	1.75	36.4	4	17.3	1.63	41.1	4	22.0	2.33	42.9			
	1929	Summer	4	19.0	1.46	46.2	4	17.0	2.38	43.4	4	25.0	2.11	44.7			
	1930	do	4	13.5	1.95	48.8	4	16.5	1.50	48.9	4	22.0	2.50	52.3			
Jersey	1929	Winter	3	23.7	1.55	35.6	3	31.0	1.90	39.8	3	35.2	1.73	43.4			
	1930	do	2	12.0	1.50	35.0	4	18.0	1.36	41.6	4	14.5	2.28	42.0			
	1929	Summer	4	17.0	1.09	39.6	4	16.0	1.94	47.7	3	18.0	1.90	50.0			
	1930	do	4	9.7	2.10	50.0	4	22.1	2.42	51.8	4	25.2	2.65	52.4			

<sup>1</sup> Numerical values represent degree of bone calcification: 0, none; 1, perceptible; 2, distinct; 3, advanced; and 4, nearly complete.

## BACTERIOLOGICAL STUDIES OF GOAT'S MILK

Although bacterial flora of aseptically drawn cow's milk has been extensively investigated (7), aside from the contribution in relation to undulant fever the udder flora of goat's milk has received little attention. The purpose of this investigation was to determine (1) the normal bacterial count of goat's milk produced under properly controlled dairy-farm conditions, (2) the average number of microorganisms contributed by the does to this total under practical conditions of milking, and (3) the kinds of bacteria and number of each in aseptically drawn goat's milk.

Only healthy animals, fairly well along in lactation and having normal udders, were used in these studies. The milk was handled under such approved methods as would ordinarily be found in any small dairy, which included steam sterilization of equipment and utensils besides adequate cooling and storage facilities for the milk. Milking was done by hand, proper sanitary precautions both as to the animals and the milkers being observed. In making the bacterial counts, the procedure outlined in Standard Methods of Milk Analysis (5) was followed, except as noted.

For a 27-week period beginning May 9, 1929, daily bacterial counts were made of 24-hour-old bottled mixed milk from a herd of 35 milking does of the Saanen and Toggenburg breeds. In addition, bacterial counts of samples of the complete milking of each of these individual does, the samples being taken directly from the milking pails, were made once weekly. These latter samples were plated within an hour after milking.

The procedure was somewhat altered in the case of aseptically drawn milk, samples of which were obtained on August 19 and September 12, 1932, from each doe in the milking herd. In such instances the does were prepared for milking by washing the udders with soap and water, rinsing, drying, and finally applying a 67-percent alcohol solution to each teat. Samples were obtained from the strippings of the afternoon milking. The milk was drawn by hand into sterile test tubes, one tube being used for each teat and care being exercised to avoid contamination. The samples were stored in the ice box overnight, and 1 cc of each milk sample plated directly on tryptophan-peptone-yeast extract agar the following day. In a few cases of milk with high counts, a higher dilution was found to be necessary and in certain instances the 1-cc portion was divided between two plates. Incubation at 37° C. showed no advantage over room temperature (25°), and 3 days' incubation as a rule produced as many large colonies as did longer incubation periods of 1 to 2 weeks.

Samples of milk failing to show any initial growth were incubated for an additional week and again examined for the presence of bacteria. The procedure included centrifuging, staining, and the making of fresh agar slant cultures.

Burri (27) in 1928 proposed the use of milk smears in place of plates as a simple and convenient means of evaluating total bacterial counts, and Dorner (39) pointed out the greater accuracy of the count by the former method owing to the carrying over, to the agar slant, of milk nutrients essential for rapid and immediate growth. Because of the small numbers of organisms encountered in goat's milk, this method was less satisfactory than the direct plating of 1-cc

quantities of milk. At the same time it was felt that the advantages due to a carry-over of milk nutrients as advocated by Dörner were accomplished in the technique as outlined by the use of this larger inoculum.

Results of the bacteriological examination of bottled goat's milk revealed an average daily count, for the entire 27-week period, of 1,340 bacteria per cubic centimeter. The lowest count was 200 and the highest 9,800 bacteria per cubic centimeter of milk; 80 percent of all counts were considerably less than 1,000 bacteria and 52 percent were less than 500 bacteria per cubic centimeter.

Individual samples, numbering 728, obtained at weekly intervals from the does supplying the bottled milk during the 27-week period produced an average number of 775 bacteria per cubic centimeter of milk, with a minimum and maximum count of 10 and 100,000 bacteria per cubic centimeter, respectively. The average results indicate that the does probably contributed more than 50 percent to the average bacterial count of the milk that was bottled.

TABLE 19.—Results of bacteriological examination of the strippings of aseptically drawn milk from Saanen and Toggenburg goats of different ages and stages of lactation

SAANEN BREED								
Goat No.	Age	Samples taken Aug. 19				Samples taken Sept. 12		
		Stage of lactation	Daily milk production	Bacteria count per cubic centimeter of milk from—		Daily milk production	Bacteria count per cubic centimeter of milk from—	
				Right test	Left test		Right test	Left test
	Years	Days	Pounds	Number	Number	Pounds	Number	Number
404 <sup>1</sup>	6	454	6.0	80	24	4.3	500	10
423 <sup>2</sup>	6	185	5.8	0	0	4.2	0	0
412	6	180	3.9	0	0	3.1	0	0
653	2	179	2.7	4	4	2.3	0	0
292	7	177	6.5	0	1,000	3.9	0	1,500
39957 <sup>3</sup>	1	176	3.5	0	10	2.0	0	0
614	3	160	9.2	2	0	4.5	0	0
466	4	156	6.7	0	0	5.0	0	0
416	6	149	4.8	1	0	2.5	0	0
498	3	105	8.9	2,000	3,000	5.9	10,000	16,000
255	10	94	4.9	0	2,000	2.4	0	20,000

TOGGENBURG BREED								
751	2	182	2.1	0	7	1.2	0	800
570	4	181	6.3	0	0	3.3	0	4,000
706	3	180	4.8	0	500	3.7	0	9,000
367	8	179	3.8	150	0	4.1	100	0
708 <sup>4</sup>	3	171	3.5	200	2,000	0	0	0
717	3	157	5.3	0	0	3.3	0	0
744 <sup>5</sup>	2	120	.8	7	0	0	0	0
745	2	116	4.2	0	0	4.0	0	0
747	2	113	4.6	1,000	0	3.5	600	0
508 <sup>6</sup>	6	113	4.2	0	0	3.4	0	0
3763	8	110	5.1	0	400	2.6	0	2,500
748 <sup>7</sup>	2	98	2.9	0	0	0	0	0

<sup>1</sup> Milked through from former lactation period without kidding.

<sup>2</sup> Had no left test.

<sup>3</sup> No sample taken at second test.

<sup>4</sup> Sick at time of second test.

<sup>5</sup> Died up before second test.

<sup>6</sup> Had no right test.

<sup>7</sup> Died suddenly before second test.

Eighty samples of milk from 11 Saanen and 12 Toggenburg does were included in the studies of aseptically drawn milk. The age of these does, their stage of lactation, quantity of milk produced, and the bacteria count per cubic centimeter are shown in table 19. Milk from 49 teats produced no evidence of bacterial growth. In the remaining 31 samples, from 13 right and 18 left teats, 3 organisms were isolated and identified according to Hucker's (72) classification. These results are shown in table 20. The organisms appeared in pure culture except in goat 706, in which *Micrococcus epidermidis* and *M. aurantiacus* were found in the same teat. *M. epidermidis* represented 87 percent, *M. aurantiacus* 11 percent, and *M. varians* 2 percent of the total number of bacteria encountered.

TABLE 20.—Extent of presence of different types of bacteria isolated from aseptically drawn goat's milk August 19 and September 12

Breed of goat	Teats examined	Teats with organisms	<i>Micrococcus epidermidis</i>		<i>Micrococcus aurantiacus</i>		<i>Micrococcus varians</i>	
			Teats involved	Organisms per cubic centimeter of milk	Teats involved	Organisms per cubic centimeter of milk	Teats involved	Organisms per cubic centimeter of milk
	Number	Number	Number	Number	Number	Number	Number	Number
Saanen.....	40	17	14	3, 830	2	752	1	1, 000
Toggenburg.....	40	14	12	1, 147	2	3, 500	1	500
Total or average.....	80	31	26	2, 592	4	2, 126	2	750

The data presented in table 19 show an average count of 2,500 bacteria per cubic centimeter of milk, from the 31 teats, showing evidence of microbial life. Several of the does produced milk of high bacterial count in at least one teat, whereas goat 608 showed a consistently high count in both teats. The high bacterial content of the milk obtained from these several teats, when the milk was aseptically drawn, is not significant evidence of a generally high bacterial count of goats' milk produced under normal conditions, since every effort was made to cultivate these organisms and since the last portion of the milking, which was used in this test, was found to carry more organisms than either the foremilk or the middle milk. In spite of these unfavorable conditions, however, calculations based on the actual number of bacteria contributed from each doe's milking to the total quantity of milk produced, show that aseptically drawn milk from the herd studied had an average count of 960 bacteria per cubic centimeter. This number is comparable with the actual number found over a much longer period reported previously.

*Micrococcus epidermidis* was by far the most widespread organism and occurred in the largest numbers. The reason for this finding may be the degree of natural resistance possessed by this organism and the fact that it is often found in the deeper layers of skin glands. None of the bacteria isolated appear to have pathological significance. Neither was there evidence that the age or breed of the doe, nor the quantity of milk or stage in lactation, were factors affecting the presence of micro-organisms in the milk.

Among the most significant results of these udder studies is that more than 60 percent of the teats examined failed to show evidence of the presence of bacteria in spite of the fact that the media used provided an abundant source of both carbon and nitrogen for the general growth requirements of bacteria. This result was verified by a careful microscopic examination of centrifuged samples. The freedom of so large a proportion of teats from the presence of bacteria largely explains the low bacterial count of the bottled milk and offers additional evidence for assuming that a count of not more than 2,500 bacteria per cubic centimeter is a reasonable requirement for market goat's milk.

## FEEDING COMPARISONS OF THE MILKS

### KIDS AS TEST ANIMALS

The comparative feeding experiments with kids consisted of two series. In the first, the animals were fed raw or boiled milks without supplements for measurement of the growth rate and changes in the hemoglobin level of the blood. In the second series, groups were fed iron, and iron and copper, added to the milk to determine their effect in the prevention or alleviation of nutritional anemia and other manifestations of malnutrition.

Healthy Toggenburg or Saanen kids born late in the winter or early in the spring were used for the milk-feeding work. These kids were placed in the experimental feeding pens when they were between 1 and 2 weeks of age. The pens were built with wooden sides and concrete floors. Wood shavings, renewed at least once each week, were used as bedding. In some instances, two kids were placed in a single pen. In selecting experimental animals no consideration was given to breed or sex. All male kids were castrated a few days after birth. Each animal was weighed at the beginning of the experiment and during each succeeding week. Blood samples were taken from the neck vein of each kid before it was placed on experiment, at the end of 2 weeks, and every 4 weeks thereafter for hemoglobin determinations made by the Newcomer method (98).

The kids were bottle-fed at 8 a. m., 12 m., 4 p. m., and 12 p. m. The animals in any given experiment received equal quantities of milk each day, the quantity being regulated by the group consuming the least. In the usual case this was the group fed Jersey milk. Change in level was made weekly as indicated by the readiness with which the milk was consumed during the previous week. The total nutrients (protein + lactose + 2.25 times fat) consumed by each animal were computed weekly from the chemical analyses of the milks, and these data, together with individual weekly gains, were used as the basis for comparisons. Weight and hemoglobin data for comparative purposes were obtained at the same time from normal kids selected for breeding stock and fed the regular station ration of hay and grain, ad libitum, in addition to milk to a maximum of 40 ounces. These animals constituted the control group.

In the first series of experiments, during the summer months of 1930 and 1931, 18 kids received equal quantities of an exclusive raw-milk diet for 26 consecutive weeks. The kids fed Jersey milk made more rapid weekly gains than those receiving either goat's or Holstein milk. However, there appeared to be no significant difference

in these gains when compared on an equal nutritive basis (fig. 8). Results were found to be similar for each year. Feeding eight kids these same milks after boiling for 1 minute produced results similar to those obtained by feeding raw milk.

There was a uniform gain in weight per pound of total nutrients consumed up to the time that about 30 pounds of gain had been made.

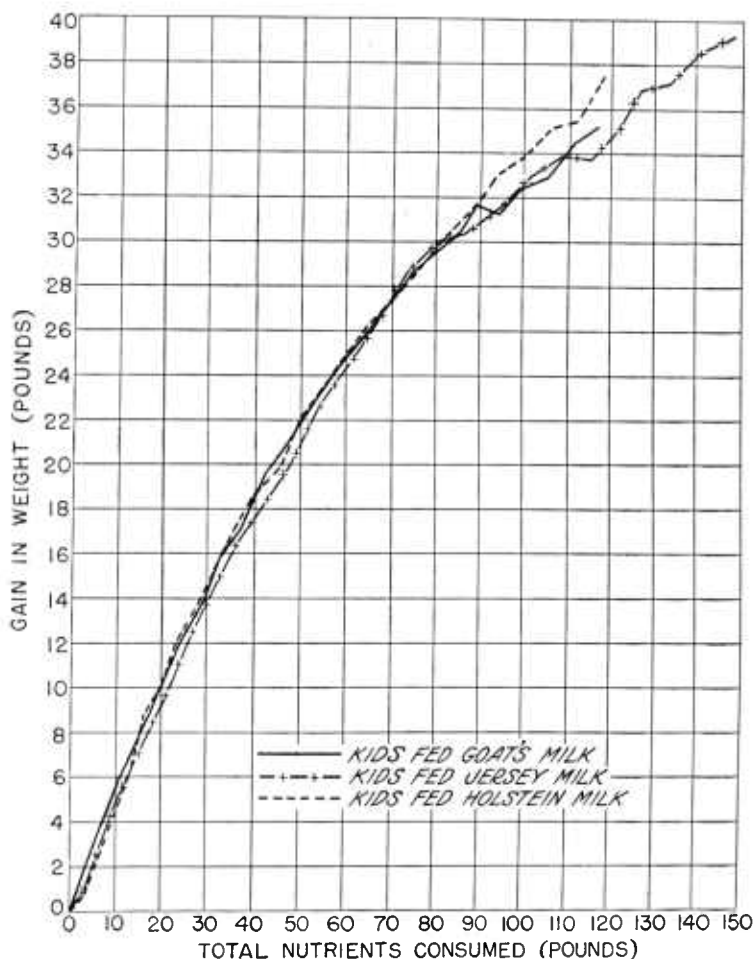


FIGURE 8.—Comparison of gains in weight made by kids fed, for 26 weeks, an exclusive raw-milk diet of goat's milk (Saanen and Toggenburg combined) and of Holstein and Jersey cow's milk.

A gain of about 30 pounds required approximately 80 pounds of total nutrients in the form of milk regardless of the source of the milk or whether it was fed raw or after boiling. In table 21 are presented data covering this period of the milk-feeding experiment. Data were taken from the week at the end of which the nearest to an average of 30 pounds of gain in weight had been made. The control animals

reached the weight sooner than the kids fed exclusively on any of the milks.

TABLE 21.—*Gains in weight per pound of nutrient fed and average hemoglobin values of the blood of kids at the time of reaching about 30 pounds of total gain on raw and boiled goat's milk (Saanen and Toggenburg combined), Holstein, and Jersey milk*

Diet fed	Kids fed	Period fed	Average total gain	Gain per pound of nutrient fed	Average hemoglobin per 100 cubic centimeters of blood at—	
					Beginning of experiment	End of experiment
Raw milk:	<i>Number</i>	<i>Weeks</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Grams</i>	<i>Grams</i>
Goat.....	9	19	29.48	0.3691	8.46	4.62
Holstein.....	4	20	30.35	.3680	10.50	4.71
Jersey.....	5	16	30.02	.3727	9.74	4.31
Boiled milk:						
Goat.....	3	19	30.35	.3961	7.75	3.93
Holstein.....	2	20	29.92	.3660	8.50	4.90
Jersey.....	3	17	30.55	.3785	7.80	4.67
Milk, hay, and grain (controls).....	12	15	30.9	-----	9.04	8.77

The period of uniform gain per pound of total nutrients consumed was followed by a period of relative fluctuations in these values, as indicated in figure 8. At this time the milk-fed animals were anemic. Their average hemoglobin values had dropped more than 50 percent, which fact greatly impaired their appetite. Milk consumption fell off and in many cases complete refusals took place. The result was a loss of body weight, compensated for in part by a slight rise in hemoglobin level, resulting in the ingestion of more milk, which in turn increased the body weight. With the increase in weight, hemoglobin values declined and the hemoglobin-growth cycle began over again. The effect of such a procedure on the efficient utilization of the nutrients present in the milks is shown in the latter part of the curves in figure 8. These show that for a total gain of 35 pounds, each pound of nutrients consumed produced an average gain of 0.305 pound for all three milks, as compared with an average gain of 0.370 pound per pound of nutrient at 30 pounds of total gain. Such results cannot be attributed to a flattening out of the normal growth curve of kids, since the control animals reached 35.6 pounds in weight at 18 weeks of age, thereby continuing the same rate of gain as for 15 weeks.

Nutritional anemia developed on the continued exclusive feeding of any of the three milks. The rate of development appeared to depend largely on the rate with which growth took place. It is apparent, therefore, that the hemoglobin-forming properties of the body do not keep pace with the demands occasioned by a rapid increase in body weight.

In the second phase of the milk-feeding experiment, 15 kids made anemic by an exclusive diet of milk were subsequently fed the various milks with and without mineral supplements. Iron and copper were supplied in the form of ferric chloride and copper sulphate without special purification. Iron dosages were so administered that each animal received 15 mg daily, and when copper also was given the daily dosages included 1.5 mg of this mineral in addition to the 15 mg

of iron. The experiment was conducted for 5 weeks. A comparison was also made of the efficiency with which gain in weight took place during the 5-week experimental period and the preceding 5-week period of anemia development. Results are shown in table 22.

TABLE 22.—Gains in body weight and increase in hemoglobin of the blood of anemic kids fed milk of goats (Saanen and Toggenburg combined) and of Holstein and Jersey cows for 5 weeks with and without iron or iron and copper supplements

Diet fed	Animals fed	Average gain in weight	Increase in hemoglobin per 100 cubic centimeters of blood	Gain in weight per total nutrients consumed during—	
				5 weeks prior to experiment	5 weeks of experiment
	Number	Pounds	Grams	Pounds	Pounds
Goat's milk:					
Alone.....	1	4.20	0.55	0.144	0.165
With iron.....	2	9.35	2.80	.112	.272
With iron and copper.....	2	8.40	2.40	.151	.246
Holstein milk:					
Alone.....	3	4.0	.35	.165	.105
With iron.....	1	9.1	3.00	.097	.290
With iron and copper.....	1	10.5	2.10	.000	.336
Jersey milk:					
Alone.....	1	6.6	.60	.223	.189
With iron.....	2	8.65	1.93	.178	.216
With iron and copper.....	2	10.8	1.82	.144	.278

These data show a general response to the inclusion of supplements to the milks as measured by increases in hemoglobin and gains in weight made by the animals. The addition to cow's milk of iron in combination with copper produced greater gains more efficiently than did iron alone. In goat's milk, iron alone was equally as effective as iron and copper together. In all cases, however, as the hemoglobin values rose during the experimental period milk was consumed with greater efficiency by the animals receiving the supplements as measured by their gain in weight per total nutrients consumed. No such increased utilization took place during this period, or during the 5-week period prior to such therapy, with the animals receiving the milks without supplement.

It appears that low hemoglobin values resulting from an exclusive milk diet are accompanied in the kid by a loss in efficiency of food utilization that may be corrected by supplementary iron and copper feedings.

#### RATS AS TEST ANIMALS

Two series of comparative feeding experiments were also conducted with young rats. The first series dealt primarily with the hemato-poietic properties of the three milks and the second with the growth-promoting properties. The rats used in the feeding experiments were confined, at the age of 28 days, in individual cages with raised screen bottoms. Distilled water was provided. The milk was measured daily into porcelain bowls. The inorganic salt supplement when fed was added as a solution to the milk. The milk consumption was recorded daily and the body weights were taken weekly. As in the vitamin work, rats of the same litter and sex were generally arranged in triplicate, one on each of the three kinds of milk.

## ANEMIA-PRODUCING PROPERTIES OF THE MILKS

In the first series of experiments the comparative anemia-producing properties of the three milks were studied, including a comparison between winter and summer milks. Rats were divided into three experimental groups: (1) Those rats made anemic by the continued feeding of an exclusive raw-milk diet for 20 weeks; (2) those rats made anemic by feeding an exclusive raw-milk diet for 10 weeks, at which time the milk was supplemented with 0.5 mg of ferrie chloride and 0.05 mg of copper sulphate per day as a curative therapy for the next 10 weeks; and (3) those rats in which anemia was prevented by feeding the iron and copper supplements in addition to raw milk for 20 weeks. In the first and third groups the experiments were terminated after 20 weeks of feeding. At this time selected animals were killed and their entire bodies analyzed for total ash and iron, the latter being determined by the Kennedy method (77). In the second group the animals were continued on the diet of milk, iron, and copper for 25 to 29 weeks before being killed. The purpose was to determine whether any marked changes in hemoglobin content as well as other manifestations of anemia were likely to occur during such a long test period with animals that had once been anemic.

Milks produced during February, March, and April were considered winter milks, and May, June, July, and August were considered summer months of milk production. Each experimental group consisted of at least two series of rats, one placed on experiment in February and the other in June. Since 10 weeks was usually sufficient to develop severe anemia, the rats placed on experiment in February had well advanced cases of this disease by the time the cows and goats were turned on pasture. The second 10-week feeding period of this group thus provided an opportunity to observe any pronounced differences in anemia-producing properties of the three milks that could be attributed to pasture feeding of the goats and cows. The progress of anemia was followed by means of blood readings, milk consumption, and weight of the rats.

In making the hemoglobin studies, blood was obtained from the tip of the rat's tail, and hemoglobin readings were determined colorimetrically by the Newcomer method (98). Readings were made at the beginning of the experiment and at intervals of 3 to 4 weeks thereafter.

Figure 9 shows that the exclusive feeding of whole raw milk from any of the three sources led to the rapid development of nutritional anemia. In the three experiments the hemoglobin values in 1929 were determined at the end of the ninth week, and in 1930 at the end of the tenth week. In experiment 1, in the tests made in 1929, the hemoglobin values of the blood of the rats averaged approximately 11 g when milk feeding was begun. From this point, the hemoglobin values dropped steadily until at the end of the ninth week the animals usually showed characteristic symptoms of anemia. In 1930 the young rats when placed on the milk diet had an average hemoglobin value of 12.8 g. The drop in hemoglobin during the first 10 weeks on winter milk was fully as rapid as in 1929, but on summer milk the decline was interrupted after the fifth week.

The feeding tests conducted during 1929 with whole milk alone showed a noticeable relationship between volume of milk consumed and hemoglobin content of the blood (table 23 and fig. 9). In view of the fact that there was no pronounced difference in iron and copper

contents of the three milks, as shown in table 1, it appeared reasonable to suppose that increasing consumption of milk furnished increasing supplies of the elements necessary for hemoglobin formation. This relationship was not borne out in the results of the following year. In the group placed on test in February 1930, the rats on Holstein milk were the most anemic by the end of 10 weeks, even though they had consumed the most milk. However, they showed an increase in hemoglobin content during the second 10-week period.

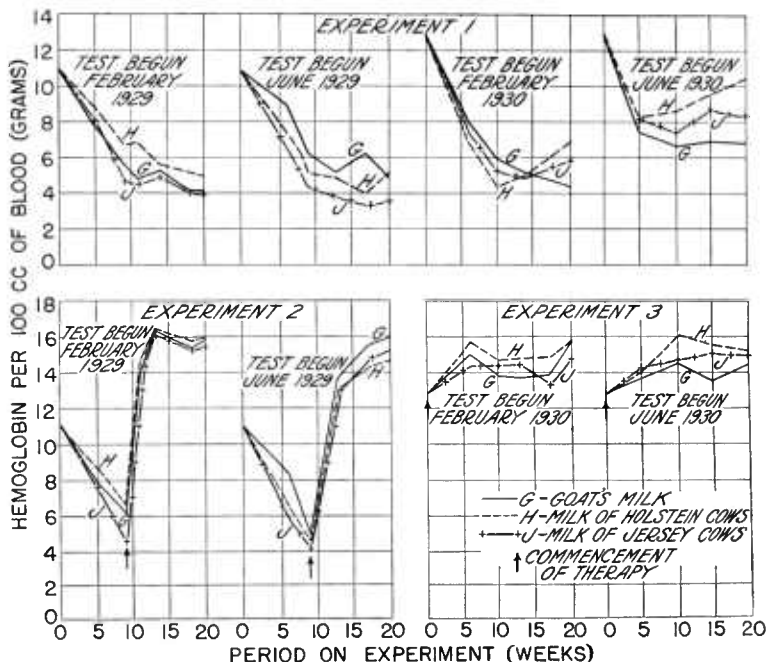


FIGURE 9.—Changes in hemoglobin content of the blood of rats fed exclusively on milk (experiment 1), on milk with iron and copper added after the tenth week (experiment 2), and on milk with iron and copper added from the beginning (experiment 3).

They received milk produced on pasture during the latter part of this period, which corresponded in time to the first 10-week period of the animals placed on experiment in June. In the group of rats placed on experiment in February, slight recovery during the last 10 weeks by the rats on Holstein and on Jersey milks is in harmony with the less abrupt decrease in hemoglobin content of the blood in the case of the group placed on experiment in June.

In general the data do not indicate an outstanding difference in the three milks so far as effect on hemoglobin content of the blood is concerned. The average hemoglobin values at the close of the first 9- and 10-week periods in experiments 1 and 2 were 5.64 g for the 27 rats fed goat's milk, 5.91 g for the 27 rats fed Holstein milk, and 5.06 g for the 26 rats fed Jersey milk. These values are in the same order as the quantity of milk consumed. In other words, severity of anemia was associated with decrease in volume of milk consumed.

TABLE 23.—Average gain or loss in weight and average milk consumption of rats fed goat's milk (Saanen and Toggenburg combined) and Holstein and Jersey milks, to determine their anemia-producing properties

## EXPERIMENT 1 (DIET OF MILK ONLY)

Date of beginning of experiment	Source of milk	First 10 weeks of feeding period					Second 10 weeks of feeding period				
		Rats at beginning of experiment	Initial weight	Gain in weight	Milk consumption		Rats at beginning of experiment	Gain in weight	Milk consumption		Gain per— 100 cc of milk
					Cubic centimeters	Calories			Cubic centimeters	Calories	
February 1929	Goat.....	5	44	63	2,999	1,963	15	—	2,749	1,668	—
	Holstein.....	5	46	82	3,380	2,261	15	37	3,767	2,620	0.98
	Jersey.....	5	48	76	2,028	2,135	16	25	2,744	2,142	1.17
June 1929	Goat.....	4	52	65	3,327	1,980	4	14	3,934	2,378	—
	Holstein.....	4	52	79	3,474	2,358	4	—	3,024	2,365	—
	Jersey.....	4	52	63	2,375	2,060	3	20	2,486	1,790	1.12
February 1930	Goat.....	5	52	90	2,356	1,897	5	35	3,044	1,961	—
	Holstein.....	5	52	80	2,823	2,763	5	38	4,050	2,488	1.41
	Jersey.....	3	52	125	4,821	2,778	4	13	2,896	2,437	2.38
June 1930	Goat.....	4	53	115	4,461	2,814	4	20	4,741	2,696	—
	Holstein.....	4	55	108	4,469	2,924	4	26	3,878	2,765	—
	Jersey.....	4	55	108	2,906	2,466	4	11	3,213	2,452	—

## EXPERIMENT 2 (MILK WITH IRON AND COPPER ADDED AFTER TENTH WEEK)

Date of beginning of experiment	Source of milk	Rats at beginning of experiment	Initial weight	Gain in weight	Milk consumption	Calories	Rats at beginning of experiment	Gain in weight	Milk consumption	Calories	Gain per— 100 cc of milk
February 1929	Goat.....	5	45	62	3,120	1,931	5	46	3,926	2,398	1.15
	Holstein.....	5	48	70	2,923	2,161	5	70	4,705	3,275	1.49
	Jersey.....	4	47	59	2,637	2,103	4	70	4,004	3,249	1.75
June 1929	Goat.....	4	48	59	2,875	1,748	3	86	5,202	2,845	1.08
	Holstein.....	4	49	75	3,306	2,246	3	96	4,878	3,785	1.74
	Jersey.....	3	54	95	3,129	2,512	3	20	4,878	3,785	1.97

## EXPERIMENT 3 (MILK WITH IRON AND COPPER ADDED FROM THE BEGINNING)

Date of beginning of experiment	Source of milk	Rats at beginning of experiment	Initial weight	Gain in weight	Milk consumption	Calories	Rats at beginning of experiment	Gain in weight	Milk consumption	Calories	Gain per— 100 cc of milk
February 1930	Goat.....	5	50	74	3,215	2,284	5	61	4,635	2,932	1.32
	Holstein.....	5	43	95	3,620	2,268	5	52	4,869	2,982	1.07
	Jersey.....	5	47	120	3,323	2,668	4	48	3,704	3,108	1.26
June 1930	Goat.....	4	51	139	5,268	3,343	4	20	5,339	3,339	—
	Holstein.....	4	57	141	4,832	3,012	4	20	5,582	3,152	—
	Jersey.....	4	55	153	3,664	3,123	4	25	4,211	3,182	—

1 2 rats died during the feeding period; data based on survivors.

2 4 rats died during the feeding period; data based on survivors.

3 1 rat died during the feeding period; data based on survivors.

A study was made of the data on gains in weight, consumption of milk, and gains per 100 calories of milk consumed by the rats receiving milk exclusively for the first 10-week feeding period. The summarized results of this study showed the mean values given in table 24.

TABLE 24.—*Relative milk consumption and gain in weight made by rats fed exclusively on milk of goats (Saenens and Toggenburgs combined) and of Holstein and Jersey cows for 10 weeks*

Source of milk	Rats fed		Average quantity of milk consumed by—		Average gain in weight			
	Females	Males	Females	Males	Total by—		Per 100 calories by—	
					Fe-males	Males	Fe-males	Males
					Grams	Grams	Grams	Grams
Goat.....	15	12	2,047	2,035	66	75	3.19	3.57
Holstein.....	15	10	2,273	2,283	84	89	3.69	3.86
Jersey.....	15	10	2,249	2,282	80	90	3.52	3.88

A variance analysis on the intake of calories showed that after the removal of variances due to factors other than the milks, the odds were greater than 99 to 1 that the milks were consumed at significantly different caloric levels. The variance analysis on gains in weight gave essentially the same results as that for calories of milk constituents consumed. Finally, the variance analysis on gain per 100 calories of milk showed a greater difference between sexes than between milks, although neither were sufficiently great to be of significance since the odds were somewhat less than 19 to 1.

The nature of these results prompted the application of covariance analysis in order to remove the effect of the variations in consumption of milk from the gains in weight. The results are given in table 25. Such an analysis with comparison of gain to feed consumption has been made recently by Crampton (31) on data of a similar nature.

TABLE 25.—*Analysis of gains corrected for caloric intake*

Item	Degrees of freedom	Sum of squares	Mean square	Item	Degrees of freedom	Sum of squares	Mean square
Between sexes.....	1	1,086.1	1,086.1	Replications.....	26	9,353.3	359.7
Between milks.....	2	506.7	253.4	Error.....	48	6,434.6	134.1

The ratio of the items "between milks" and "error" is 1.89, which is outside the 0.05 limit of significance for the degrees of freedom as indicated in table 25. The confirmation afforded here for lack of significance in the differences in gains due to corresponding differences in the intake of calories is strongly convincing that no pronounced differences existed among the milks which could have influenced the efficiency of the gains. The question remains unanswered concerning the reason for the limited consumption of goat's milk, especially since the wide difference in caloric content per unit volume between Holstein and Jersey milk did not prevent the rats from adjusting their intake proportionally.

In experiment 2 the addition of iron and copper supplements to the milk diet of rats at the end of the tenth week led to a rapid rise in the hemoglobin content of the blood. In the test begun in 1929, in 15 days after the addition of supplements the hemoglobin value had risen to more than 15 g per 100 cc of blood, at which it remained until the end of the experiment at 20 weeks. Approximately this same hemoglobin value was reached in the test begun in the summer of 1929, although the rise was not so rapid as in that of the former test.

Consumption of goat's milk, although it exceeded that of Jersey milk a part of the time and frequently nearly equalled that of Holstein milk, was not sufficient to furnish the same caloric intake as the cow's milk. As a result, in all cases the gains were lowest on goat's milk. However, the use of iron and copper definitely increased the consumption of milk and the growth of the rats on all three milks from the tenth to twentieth weeks as compared with the milk consumption and growth of rats used in experiment 1, which were similarly fed except that they did not receive the iron and copper supplements.

In experiment 3, the inclusion of the iron and copper supplements in the milk diet throughout the entire feeding period enabled the rats to build up and maintain a high hemoglobin level. The first hemoglobin determinations were made during the fifth week of the experiment, when values approximating 14 g per 100 cc of blood were found. About the same level was maintained throughout the remaining 15 weeks of the experiment. Several of the hemoglobin determinations for the animals fed Holstein milk were more than 15 g.

The rats placed on experiment 3 in February and fed goat's milk gained rapidly until the early part of April, when they ceased growing for a time. Meanwhile, the rats on Holstein and Jersey milk continued to grow during April and May. The subsequent resumption of growth of those rats on goat's milk, which coincided with the use of pasture-produced milk, was sufficiently rapid to enable them to reach weights nearly equal to those on Holstein and Jersey milks. In the test begun in June and carried through the summer and fall months, the gains on all three milks were especially rapid during the first 10 weeks. A comparable rapid growth, especially of the rats fed goat's milk, was made by the rats fed milk without iron and copper supplement (experiment 1).

The results of experiment 3 emphasize the important role played by the palatability factor. Gains were generally proportional to the consumption of milk, particularly as measured by the caloric intake. The results for the first 10 weeks suggest a somewhat lower efficiency of utilization for the goat's milk, as measured by the gain of the rats per unit caloric intake. The entire 20-week period shows the same tendency, although less marked.

#### IRON CONTENT OF THE RATS USED IN EXPERIMENTS 1, 2, AND 3, AND ITS RELATION TO THE BLOOD FINDINGS

Smythe and Miller (116) found that the bodies (minus the alimentary tract) of rats at the age of 6 weeks contained 0.0045 percent of iron, a level maintained by the growing rats until maturity, after which the iron content increased to 0.0051 percent and remained constant at that level. Analyses for iron content on animals from the three experiments already discussed are shown in table 26. These results substantiate the findings obtained by the hemoglobin deter-

mination as indicated in figure 8. The animals in experiment 3 had the highest iron content, the values being equivalent to those obtained for normal rats by Smythe and Miller. The results of experiments 2 and 3 indicate that the body can be depleted to approximately 0.003 percent without being deprived of the necessary iron for hematopoiesis. This leads to the belief that iron is converted primarily into hemoglobin until the normal is attained and the excess iron is then stored in the tissues.

TABLE 26.—*Summary of data on iron content of rats used in experiments 1, 2, and 3*

Experiment No. <sup>1</sup>	Source of milk fed	Rats used	Length of feeding	Age of rats	Empty body weight	Ash content of body	Iron content of body	Hemoglobin per 100 cc of blood
		Number	Weeks	Weeks	Grams	Percent	Percent	Grams
1	Goat	7	20	24	152	4.10	0.00235	5.78
	Holstein	7	20	24	169	3.73	.00205	7.17
	Jersey	7	20	24	173	3.53	.00195	6.20
2	Goat	4	36	40	195	3.42	.00326	16.00
	Holstein	4	35	39	194	3.71	.00362	15.83
	Jersey	4	39	43	179	4.01	.00301	15.44
3	Goat	7	20	24	185	4.62	.00458	16.55
	Holstein	9	20	24	188	3.85	.00464	15.27
	Jersey	9	20	24	209	3.10	.00420	15.42

<sup>1</sup> For a description of the experiments, see p. 50.

#### GROWTH-PROMOTING PROPERTIES OF THE MILKS

In the second series of comparative feeding experiments with young rats, a study was made of the growth-promoting properties of the three milks as measured by gain in weight and economy of food utilization. Three different feeding conditions formed the basis for making comparisons in this series. In the first an attempt was made to control the protein intake of the milks; in the second an attempt was made to control the milk consumed at specified volume levels; and in the third the effect of boiling the milks was studied.

In the work on growth and economy of food utilization of rats, the protein intake of the milk from goats (Saenens and Toggenburgs combined) and from Holstein and Jersey cows was equalized as far as possible in order to avoid the variable milk consumption and the possible influence that this variation had on the gains of the rats in the previous experiments. Three sets of eight male rats each were fed raw milks at levels that provided approximately an equal quantity of protein from each kind of milk. This quantity was calculated from the analysis of the weekly milk sample of the preceding week. The quantity of milk was regulated so that all rats consumed all the milk given them each day. Prophylaxis against anemia was provided by the daily administration of 0.5 mg of iron and 0.05 mg of copper, as in the previous experiment. At the end of an 8-week feeding period, the animals were killed, and six rats fed each of the three milks were prepared for chemical analysis. The entire body, including all organs, with stomach and intestines cleaned, was analyzed for water, protein, fat, and ash.

The results of an 8-week feeding period on 24 male rats are shown in table 27. It will be noted that equalization of the protein intake provided a nearly equal volume of goat's and Holstein milk and an approximately equal intake of calories of goat's and Jersey milk.

Although the total gain, as well as the gain per 100 cc of milk, per 100 calories, and per 1 g of protein, is lower in the case of goat's milk than in either of the cow's milks, the differences are not statistically significant. The average body composition of the rats showed no marked difference in protein content. The rats on Holstein milk had the highest fat content, although in composition of fat the milk was not greatly different from goat's milk and was actually lower in total calories. Differences in the fat content of Holstein and Jersey milk were not reflected in the composition of the rats. The rats on goat's milk were generally lower in fat content than those on Holstein or Jersey milk. The order of decreasing fatness was related to the total gain in live weight.

TABLE 27.—Comparative growth-promoting properties of goat's milk (Saanen and Toggenburg combined), Holstein milk, and Jersey milk when fed to rats for 8 weeks on the basis of an approximate equal protein intake

Source of milk	Rats fed	Average initial weight	Average total gain	Average total consumption of—			Gain made per—		
				Milk	Calories	Protein	100 cc of milk	100 calories	Gram of protein
	Number	Grams	Grams	Cubic centimeters		Grams	Grams	Grams	Grams
Goat.....	8	59.0	79.1	3,242.8	1,962.72	96.4	2.44	4.03	0.82
Holstein.....	8	58.5	89.5	3,263.8	1,914.56	99.9	2.74	4.68	.90
Jersey.....	8	59.3	86.9	2,530.1	1,985.28	97.0	3.43	4.38	.90

Source of milk	Rats fed	Analysis of the rat bodies					
		Bodies examined	Average body weight (empty)	Composition			
				Water	Protein	Fat	Ash
	Number	Number	Grams	Percent	Percent	Percent	Percent
Goat.....	8	6	119.3	66.3	19.8	10.9	4.0
Holstein.....	8	6	131.0	62.8	20.0	13.6	3.9
Jersey.....	8	6	130.9	63.8	19.6	13.0	3.7

Under the second feeding conditions of these studies, raw goat's milk and raw Holstein and Jersey milks were fed at five graded levels of intake to compare the effects of different volumes of milk as well as different quantities of protein, fat, and total calories on the body gains and the economy of gains of rats. The quantity of milk was increased by equal intervals for all five levels. Allowance was made for an increasing rate of consumption by the rats during the fifth to twelfth week of life and for sex differences. Only Jersey milk was fed at level 1, the lowest, since at this low intake the caloric content of the goat's and Holstein milks was not sufficient for growth. Similarly, at level 5, the highest, Jersey-milk feeding was omitted since its high caloric value at this level precluded any volume comparisons.

The first week of milk feeding was treated as a preliminary or adjustment period. The summarized results on the rats cover an 8-week experimental period which extended from the age of 5 to 13 weeks. Anemia prophylaxis was given as in the previous experiment.

The gains of the rats and their milk consumption by feed levels and by sexes are shown in table 28. Some irregularities in con-

sumption occurred, particularly with the rats fed Jersey milk at levels 3 and 4. The high caloric value of this milk made it extremely difficult for the rats to consume increased quantities toward the latter end of the feeding period that were comparable with the quantities consumed by the rats fed the goat's and the Holstein milk. At all levels, however, at which the three milks were fed, the animals receiving Jersey milk consumed the most calories. Among the female rats no data are presented for the animals fed Holstein milk at level 4, since inadvertently these animals consumed much more than the required milk for this level.

TABLE 28.—*Comparative growth-promoting properties of goat's milk (Saanen and Toggenburg combined), and Holstein and Jersey cow's milk when fed to rats for 8 weeks at different levels of milk intake*

MALE RATS										
Feeding level No.	Source of milk fed	Rats fed	Average initial weight	Average total gain	Average total milk consumed		Gain per--			
							100 cc milk	100 calories	Gram protein	Gram fat
		Number	Grams	Grams	Cubic centimeters	Calories	Grams	Grams	Grams	Grams
1	Jersey	2	32	40	882	750	4.53	5.33	1.11	0.813
	Goat	3	39	57	1,738	1,161	3.24	4.91	.97	.859
2	Holstein	3	36	43	1,764	1,141	2.44	3.77	.72	.682
	Jersey	4	42	94	1,764	1,584	5.32	5.93	1.25	.905
3	Goat	3	37	111	2,753	1,749	4.03	6.35	1.34	1.08
	Holstein	3	35	99	2,647	1,666	3.74	5.94	1.16	1.06
4	Jersey	3	50	111	2,066	1,875	5.37	5.92	1.25	.90
	Goat	3	45	157	3,147	1,999	4.98	7.85	1.65	1.33
5	Holstein	1	51	120	3,012	1,884	3.98	6.37	1.25	1.13
	Jersey	2	44	159	2,585	2,229	6.15	7.13	1.50	1.07
5	Goat	2	54	167	3,709	2,533	4.50	6.59	1.38	1.12
	Holstein	3	49	152	3,584	2,217	4.24	6.85	1.34	1.21
FEMALE RATS										
2	Goat	3	38	44	1,566	980	2.81	4.49	0.93	0.770
	Holstein	3	36	47	1,554	976	3.02	4.82	.95	.898
3	Jersey	3	41	68	1,546	1,342	4.40	5.07	1.05	.775
	Goat	3	41	74	2,319	1,484	3.19	4.98	1.06	.85
4	Holstein	3	41	78	2,321	1,465	3.36	5.32	1.04	.95
	Jersey	3	51	91	1,889	1,729	4.81	5.26	1.12	.80
5	Goat	3	43	97	2,544	1,620	3.81	5.99	1.26	1.01
	Jersey	3	47	104	2,239	1,982	4.65	5.25	1.09	.80
5	Goat	1	47	108	3,066	1,978	3.52	5.46	1.16	.92
	Holstein	4	47	106	3,041	1,898	3.49	5.58	1.10	.99

In general, the total gains as well as the efficiency of the gains of the rats increased with increasing milk intake. Comparison of the three milks at the same total-consumption levels shows that the Jersey milk usually produced the greatest total gain, contained the most calories, and showed the greatest gain per unit consumption of milk. On an equal caloric-consumption basis the three milks were not consistent in their relative efficiency of gain. The same was generally true for an equal protein basis.

When the gains were plotted against milk consumption they were found to express a linear relationship. Correlation coefficients and regression lines were accordingly calculated for the relationship of volume and calories of milk consumed to gains made by the rats. The results given in table 29 show corrected correlation coefficients, which range from 0.36 to 0.95. It is noteworthy that the minimum

of these values for males was 0.86, indicating a high correlation between gains in weight and either volume or total calories of the milk. The female rats fed Jersey milk showed an exceedingly low correlation in both series. The correlations of gain in body weight to total calories are lower than correlations of gains in body weight to volume of milk. Although the number of rats in any one group is small, all the groups show essentially the same trend, and the  $r$  values are sufficiently high to show significance with the possible exception of the group of females on Jersey milk.

TABLE 29.—Correlations of gains in body weight of rats to the volume and caloric intake of milk

CORRELATIONS OF GAINS IN BODY WEIGHT TO VOLUME OF MILK

Source of milk	Sex of rats fed	$n$ (num- ber)	$r$ (correla- tion coef- ficient)	$\bar{r}$ (corrected correla- tion coef- ficient)	$\bar{S}_{y,x}$ (coefficient non-deter- mination)	$\bar{S}_{y,x}$ (standard error of estimate)
Goat.....	{Male.....	10	0.92	0.91	0.44	20.8
	{Female.....	10	.83	.80	.63	17.5
Holstein.....	{Male.....	10	.96	.95	.32	14.2
	{Female.....	10	.84	.82	.61	17.7
Jersey.....	{Male.....	11	.94	.93	.39	15.3
	{Female.....	9	.64	.57	.87	19.7

CORRELATIONS OF GAINS IN BODY WEIGHT TO TOTAL CALORIES

Goat.....	{Male.....	10	0.89	0.88	0.51	96.8
	{Female.....	10	.84	.81	.61	68.0
Holstein.....	{Male.....	10	.95	.94	.36	64.4
	{Female.....	10	.83	.81	.62	72.0
Jersey.....	{Male.....	11	.87	.86	.53	84.4
	{Female.....	9	.49	.36	.99	89.6

The regression lines were plotted in figure 10. Those on the total-volume basis show a considerable spread among the milks. On a total-calorie basis the regression lines are grouped over a relatively narrow range with no evidence of any outstanding differences for any one milk. The female rats on Jersey milk showed the greatest divergence from the general trend of the other five groups.

The effect of boiling the milk for 1 minute was studied under the third feeding condition of the growth-promoting series. This study consisted of two parts, one in which the boiled milks were fed at restricted volume levels and the other on an ad libitum basis. Anemia prophylaxis was carried on, as previously, in both cases.

Although in the first part of this study the boiled milks were fed on a controlled-intake basis, an attempt was also made to keep the calorie consumption approximately equal. This was accomplished by feeding the goat's and Holstein milks at level 3 of the preceding experiment, whereas Jersey milk consumption was at level 2. Results of an 8-week feeding period are given in table 30.

With the exception of the male rats fed boiled goat's milk the gains were in comparatively close agreement. Consumption of protein was low in the case of boiled Jersey milk owing to the comparatively low volume of milk intake, but the gain per gram of protein was next to the highest. The calculations of gain per unit of milk consumption show the highest for boiled Jersey milk. There was little consistent

difference among any of the milks in relative gain per 100 calories. Male rats made more efficient gain per unit of milk, protein, or calorie, than female rats, except in the case of Holstein milk, for which the reverse was true. Comparison with the same milks fed at the same levels in the raw state (table 28) shows a lower gain on the boiled milks. Inasmuch as the experiments with boiled milk were made approximately 8 weeks after the experiments with raw milk, a final conclusion cannot be drawn as to the relative value of boiled and raw

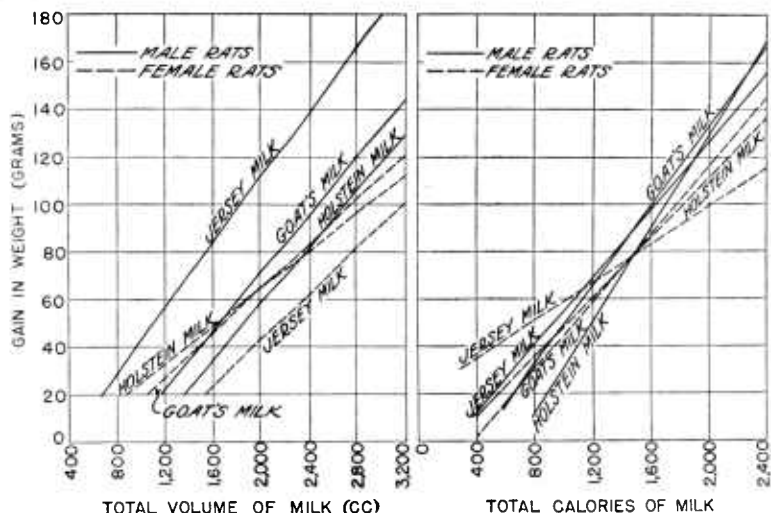


FIGURE 10.—Regression lines expressing the relationship between gains in weight of rats and the total volume and total calories in goat's milk (Saanen and Toggenburg combined) and Holstein and Jersey cow's milk ingested.

milks. The greater initial weights of the rats fed boiled milk, necessitating a larger proportion of their food intake for maintenance, undoubtedly contributed to an apparently poorer efficiency of food utilization. The data show, however, that gains of the animals in both experiments took place proportionately to the nutrients in the milks consumed.

TABLE 30.—Comparative growth-promoting properties of boiled goat's milk (Saanen and Toggenburg combined) and Holstein and Jersey milks when fed to rats for 8 weeks at controlled feeding levels

Source of milk	Feeding level No.	Rats used		Average initial weight	Average total gain	Average milk consumption			Gain per—		
		Sex	Number			Volume	Protein	Calories	100 cc milk	Gram protein	100 calories
				Grams	Grams	Cubic centimeters	Grams		Grams	Grams	Grams
Goat	3	Male	3	45.0	69.7	2,554	91.42	1,682	2.72	0.762	4.15
	3	Female	3	54.0	50.0	2,461	78.75	1,515	2.03	.635	3.41
Holstein	3	Male	2	41.0	55.0	2,646	82.55	1,781	2.08	.594	3.09
	3	Female	3	49.7	57.0	2,331	80.95	1,552	2.45	.704	3.67
Jersey	2	Male	2	53.5	57.5	1,764	77.80	1,638	3.26	.739	3.51
	2	Female	3	47.0	50.3	1,564	69.28	1,459	3.22	.726	3.45

Milk boiled for 1 minute was fed to 15 rats on an ad libitum basis for 10 weeks, and to 12 of the 15 animals for a subsequent 10-week period. Results are shown in table 31.

TABLE 31.—*Comparative growth-promoting properties of boiled goat's milk (Saanen and Toggenburg combined) and Holstein and Jersey milks when fed to rats ad libitum and with anemia prophylaxis for 20 weeks*

FIRST 10 WEEKS									
Source of milk	Rats used			Average initial weight	Average total gain	Average milk consumption		Gain per—	
	Male	Female	Total					100 cc milk	100 calories
	Number	Number	Number	Grams	Grams	Cubic centimeters	Calories	Grams	Grams
Goat.....	2	3	5	56.4	118.2	4,660	2,932	2.54	4.03
Holstein.....	2	3	5	56.0	126.8	4,655	2,767	2.79	4.69
Jersey.....	2	3	5	53.0	126.2	3,320	2,532	3.80	4.99

SECOND 10 WEEKS									
Goat.....	2	2	4	-----	27.3	5,518	3,186	0.49	0.85
Holstein.....	2	2	4	-----	40.2	5,792	3,815	.69	1.21
Jersey.....	2	2	4	-----	22.5	4,163	3,356	.54	.67

During the first 10 weeks, the rats fed goat's and Holstein milks consumed approximately equal volumes of boiled milk. These quantities were in excess of those consumed by the rats fed Jersey milk. Notwithstanding this difference in total consumption of boiled milks, the total calories consumed were fairly uniform for all three milks, thus indicating a tendency on the part of the rats to adjust consumption of milk to an equal caloric intake. Thus the rats fed Jersey milk made a better gain per 100 cc of boiled milk consumed, but on the basis of 100 calories of milk the gains were more nearly equal for all three milks.

The effect of increased age and weight of the rats in relationship to their food requirements is reflected in the second 10-week period of this feeding experiment. During this period the total boiled-milk consumption was greater in every case than during the preceding 10-week period. However, gains increased at only one-third to one-fifth of the previous rate, which fact in turn affected the gain in weight per 100 cc and 100 calories.

The present experiment was conducted at the same time as the summer series on raw milks (table 23). Since the initial weights of the rats and the anemia therapy in this experiment and experiment 3 of the raw-milk series are similar, a comparison of results can be made. In general, the rats fed raw milk showed a slightly greater increase in gain in weight and total milk consumption than similar animals fed boiled milk, yet a comparison of their relative gains in weight per unit of caloric intake showed no advantage in favor of the raw milk. Likewise no significant difference among the three milks was noted in their relative ability to produce gain on an equal caloric basis. It is also evident that no marked improvement in efficiency was produced by boiling milks as judged by these tests.

The use of whole milk—whether goat's or Holstein or Jersey cow's milk—as the only food except for the iron and copper supplements, for growing rats, has generally produced satisfactory growth for periods up to 20 weeks. The differences in gains on the milks were not abnormally great nor were they due to an unusual property of any one milk. The comparatively low efficiency of goat's milk observed on the *ad libitum* feeding was not found to a marked extent when the intake of protein or calories was equalized. Since milk, regardless of the kind, is a reasonably complete food for young mammals, the close agreement of the goat's, Holstein, and Jersey milks in producing gains proportional to the caloric content rather than the volume of milk is not surprising.

The measurements of curd tension showed that although there are wide differences in toughness of curd in raw milks from Jersey and Holstein cows and from goats, much of the difference disappears after boiling. One might expect nutritive efficiency for growth to be associated with ease and speed of digestibility, as indicated by a soft curd. The differences in curd tension as a measure of toughness among goat's and Holstein and Jersey cow's milk, or between raw and boiled milks, were not reflected in the rat-feeding experiments.

#### MILK-FEEDING STUDY WITH INFANTS

In another phase of the feeding experiments a study with healthy infants was undertaken in cooperation with Johns Hopkins University. Goat's milk (Saanen and Toggenburg combined) and Holstein and Jersey cow's milks were used as substitutes for that of the human mother in feeding work conducted at the Florence Crittenton Mission. The data reported were obtained on healthy infants fed between April 23 and December 3, 1931.

As healthy infants who required substitute feeding became available, they were placed on the different milks without distinction as to sex, birth weight, and other factors. Up to the age of 3 months, the feeding formula consisted of 1 part of milk to 2 parts of water with sucrose added at the rate of 5 g per 100 cc of milk. Each infant also received daily 2 cc of cod-liver oil and 10 cc of orange juice. At the ages of 3 and 4 months, 2 parts of milk and 1 part of water were used, and at ages of more than 4 months the milk was fed undiluted. The sugar was added as before, the quantity of cod-liver oil increased to 4 cc, and the orange juice to 30 cc at the age of 3 months or more. As already stated, the milk mixture was boiled immediately after preparation. By the addition of the cod-liver oil and the orange juice, vitamins A, C, and D were eliminated as limiting factors in the milk comparisons.

A registered nurse working under the direction of the physician in charge of the home supervised the feeding of the milks and the weighing and general care of the infants from which these data are reported.

The food consumption, expressed in calories, was calculated on a weekly basis from the volume of milk taken by the infants and the analyses for protein, fat, and sugar made on the milks. Only those infants who were fed without digestive or other disturbances over a 12-week period were included in the final summary. The 12-week period was considered by the pediatricians concerned as sufficient to show the differences, if any, among the milks. Outbreaks of intermittent diarrhea in the nursery from early in August to late in October

reduced the number of infants from which conclusions could be drawn. The diarrhea developed among infants on each of the three milks, although fewer on Jersey milk were affected than on goat's or on Holstein milk. The results presented are from data on 4 babies receiving goat's milk and 3 each receiving Holstein and Jersey milks.

Table 32 presents the average weekly consumption of milk and gain in weight of the infants studied. Based on these data, logarithmic growth curves were constructed (fig. 11) representing the relationship between the total caloric intake and gain in weight. A statistical treatment of these data fail to show any significant difference among the three milks in their ability to produce gain in weight per calorie consumed. Between Holstein and Jersey milks, the two milks with the greatest difference, the probability that such a difference is due to chance is 28 out of 100. A probability larger than 5 out of 100 is not considered significant.

TABLE 32.—*Weekly consumption and gains in weight of babies fed milk of goats (Saenens and Toggenburgs combined) and of Holstein and Jersey cows*

Week of experiment	Cumulative weekly consumption of milk of—			Cumulative average gain in weight on milk of—		
	Goats	Holsteins	Jerseys	Goats	Holsteins	Jerseys
	<i>Calories</i>	<i>Calories</i>	<i>Calories</i>	<i>Ounces</i>	<i>Ounces</i>	<i>Ounces</i>
First.....	2, 141	1, 796	2, 994	4. 93	1. 50	8. 91
Second.....	4, 264	4, 119	5, 661	5. 81	9. 25	10. 00
Third.....	6, 713	6, 477	8, 691	9. 75	12. 50	15. 66
Fourth.....	9, 036	8, 745	11, 957	13. 93	18. 08	18. 66
Fifth.....	11, 775	11, 249	15, 894	20. 19	20. 58	25. 08
Sixth.....	14, 352	13, 808	20, 376	24. 68	25. 75	33. 41
Seventh.....	17, 509	16, 511	24, 186	32. 19	27. 83	38. 25
Eighth.....	21, 210	19, 360	28, 051	34. 60	33. 91	41. 17
Ninth.....	24, 621	22, 118	32, 151	42. 25	35. 35	48. 17
Tenth.....	28, 051	25, 075	36, 306	45. 50	43. 25	52. 17
Eleventh.....	31, 589	28, 069	40, 733	46. 56	45. 83	55. 50
Twelfth.....	35, 653	31, 389	45, 142	49. 00	49. 58	58. 91

The fact that the babies fed Jersey milk made the highest gains, as shown in table 32, appears to be due to the higher consumption of food constituents, for in figure 11 it is shown that per unit of calorie intake the gain in weight of babies on each of the three milks is approximately the same. Hence, it is evident that the babies under observation gained in weight in direct proportion to the nutrients in the milk consumed by each.

This conclusion substantiates the results already given with these same milks fed to kids and rats.

## SUMMARY AND CONCLUSIONS

The goat's milk used in the study, which was carried on from 1929 to 1932, inclusive, was produced by 20 to 35 Saanen and Toggenburg does maintained at the Agricultural Research Center, Beltsville, Md. The milks of the two breeds were mixed, as information at hand indicated little difference between the breeds in the chemical composition of their milks. The Holstein and Jersey cow's milk were obtained from herds at or near Beltsville. In 1929, three animals of each breed were used. In 1930-31, the milk was obtained from 50 to 60 cows of each of the two breeds.

The goat's milk was similar to that of Holstein milk in percentage of water, protein, fat, and lactose. The goat's milk was more variable in composition than either the Holstein or Jersey milk, owing probably to the fact that the does freshened during a relatively short period late in the winter and in the spring, whereas cows freshen during the entire year. In general, the composition of the milk of the two breeds of goats used was comparable to that found in the literature.

Analyses for iron and copper showed no marked seasonal breed or species differences. The iron content was low in all samples. Small differences were noted in the results on calcium and phosphorus analyses. Holstein milk was generally the lowest, and seasonal trends showed minimum values in mid-summer.

In making the protein fractionations, one sample of human milk also was used. This study showed that one of the most marked differences among cow's, goat's, and human milks is the large quantity of casein in cow's and goat's milks as compared with the very small quantity in human milk. The high nonprotein nitrogen content of goat's milk also seems significant. A high ratio of albumin and globulin to casein was found in human milk. Likewise, a higher ratio was observed in goat's than in cow's milk.

Analyses of butterfat prepared from milk produced in March and June showed small but significant differences in fat constants among the three milks. There was a lower unsaturated acid and a higher insoluble volatile acid content in the goat's butterfat than in cow's butterfat. Among the fat acids determined, caproic, caprylic, and capric acids were found in considerably greater quantities in goat's butterfat than in cow's butterfat, whereas the reverse was true in the case of tetradecenoic acid.

The fat globules of Holstein milk were found to be 1.97 times and Jersey milk 5.53 times as large as those of goat's milk.

In surface-tension measurements, no significant differences were found among the three milks in their natural state.

The buffer capacity of the milk from the goats used in the study was very similar to that of the Jersey milk over the pH range of 5.2 to 6.4.

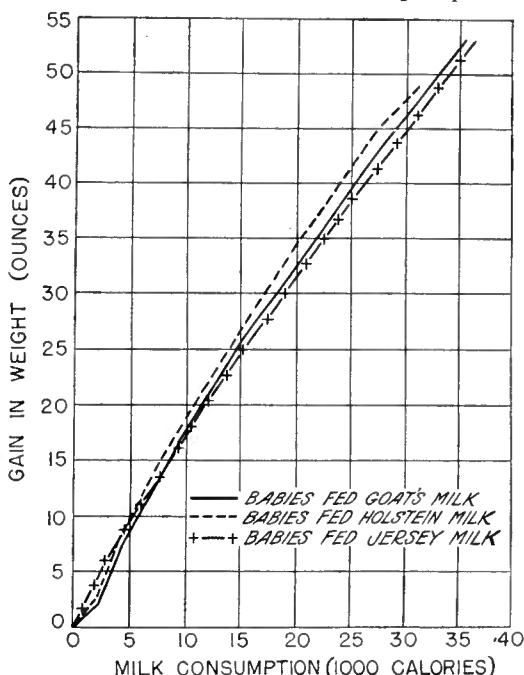


FIGURE 11.—Relationship of milk consumption to gain in weight of babies consuming goat's milk (Saanen and Toggenburg combined) and Holstein and Jersey cow's milk.

Holstein milk had a lower buffer capacity than either goat's or Jersey milk over this same range.

The milk of the Toggenburg and Saanen goats was found to be of low curd tension, as it was 31 percent softer on the average than that of Holstein milk and 54 percent softer than that of Jersey milk. Skim milks had a higher curd tension than their corresponding whole milks. Boiling either whole or skim milk reduced their curd tensions, the effect being greater in the case of skim milks. Homogenization of whole milks at 4,000 pounds' pressure produced softening of the curd but did not reduce all milk to the same level. Jersey milk was the least affected.

Fat was found to exert little influence on curd tension other than to enmesh itself within the curds, thus preventing the protein from forming an extremely hard curd. The character of the curd was found to be relatively independent of the kind or quantity of fat present in the milks and appears to be more dependent on the protein and its concentration.

Milk from individual does differed in curd tension, with the greatest number of samples being definitely soft curd. The curd-tension values varied with the stage of lactation in much the same manner as did the chemical composition of goat's milk.

The vitamin content of the milks was determined by means of feeding experiments with rats and guinea pigs. Herd milks produced during the winter and the summer for a 3-year period were studied. One cubic centimeter of goat's milk produced in the winter and 1 cubic centimeter of Holstein milk produced in either the winter or the summer furnished approximately 1 International Unit of vitamin A. Goat's milk produced in the summer contained about two-thirds of a unit per cubic centimeter. The Jersey milk produced in the winter contained  $1\frac{1}{4}$  units and that in the summer,  $1\frac{1}{2}$  units. The principal difference between the butterfats of these milks was the lower vitamin A activity of the Jersey sample of winter milk.

Goat's milk had a comparatively high potency in vitamin B and vitamin G in both winter- and summer-produced milks. Little or no difference in vitamin G was observed in the cow's milks relative to breed or seasonal and feed factors. The summer-produced cow's milk showed a slight increase from winter to summer feeding.

The vitamin C content of the milks in the quantities consumed was found to be inadequate for the prevention of scurvy in guinea pigs. The milks all varied in potency from year to year. Apparently, these variations were attributable in part at least to the feeding conditions. Although adequate summer pasturage appeared to be responsible for the increase in vitamin C potency of the milk from winter to summer during the first year of the studies, drought conditions affected the vitamin C content of the pasturage as well as the winter feeds in the two subsequent years. Milk from individual does varied in ascorbic acid content, the spring- and summer-produced milk tending to have higher contents than fall- and winter-produced milk. Exposure of the milk to air and light materially reduced its ascorbic acid content.

No marked differences in vitamin D content were noted among the three milks. The summer-produced milk generally showed a slight increase in potency over the winter-produced milk.

Goat's milk was lacking in vitamin E at the levels fed.

The goat's milk, when secreted, was found to be comparatively free from bacteria. More than 60 percent of the samples drawn from individual animals showed no evidence of bacterial growth. *Micrococcus epidermidis* represented 87 percent, *M. aurantiacus* 11 percent, and *M. varians* 2 percent of the total number of bacteria found.

The average count of bottled goat's milk prepared under normal conditions was found to be 1,340 bacteria per cubic centimeter of milk. It is felt that a total count of not more than 2,500 bacteria per cubic centimeter of milk is a reasonable standard for the production of market goat's milk.

The mixed milk of Toggenburg and Saanen goats, the Holstein herd milk, and the Jersey herd milk were fed to kids and rats under controlled feeding conditions to determine the nutritive value of goat's milk as compared with that of the other two milks. The data obtained indicated that the gains in weight took place in proportion to the total energy contained in the milks. Owing to its higher energy content, Jersey milk produced the greatest gain, per unit volume, in kids and rats. On the whole, the goat's and Holstein milks were generally consumed in greater quantities than Jersey milk when the intake was not limited. In this way the growing animals were able to adjust, in part at least, the intake of energy to their requirements even though the milks were unequal in composition. Nutritive efficiency for growth appeared not to be associated with ease and speed of digestibility, as indicated by a soft curd.

In the feeding tests with kids and rats, the development of nutritional anemia followed in time, irrespective of the type of milk fed. This was true of boiled milks as well as raw milks. It was found that anemia was cured or prevented equally well in the three milks by the addition of iron and copper. This finding substantiated the fact that each of these milks was deficient in iron.

In the kids, loss of appetite and impairment of efficiency of gain per unit of energy, causing a decrease in the rate of gain, accompanied the lowered hemoglobin values, which resulted in the early flattening out of the growth curve. The copper and iron in the dietary of kids apparently maintained the efficiency of utilization of food by preventing the onset of anemia. It was concluded that no significant difference exists in the response of kids to the ingestion of equal nutrients of goat, Holstein, or Jersey milks.

In addition to the feeding studies with kids and rats, records were kept at the Florence Crittenton Mission in Baltimore, Md., of 10 healthy babies fed the goat's and cow's milks under the direction of medical staff members of the Johns Hopkins Hospital. Each infant received, daily, in addition to milk, 2 cc of cod-liver oil and 10 cc of orange juice. These healthy babies gained in weight in proportion to the nutrients consumed in the milk. This finding substantiates the results obtained with the same milks fed to kids and rats.

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